

GENIC DIVERSITY AMONG WATER FROG GENOMES INHERITED WITH AND WITHOUT RECOMBINATION

Inaugural-Dissertation

zur Erlangung der philosophischen Doktorwürde

vorgelegt der

Philosophischen Fakultät II

der Universität Zürich

von

HANSJÜRG HOTZ

von Zürich

Begutachtet von den Herren

Prof. Dr. H. Burla

und

Prof. Dr. T. Uzzell (Philadelphia)

Zürich 1983

Zentralstelle der Studentenschaft



GENIC DIVERSITY AMONG WATER FROG GENOMES INHERITED WITH AND WITHOUT RECOMBINATION

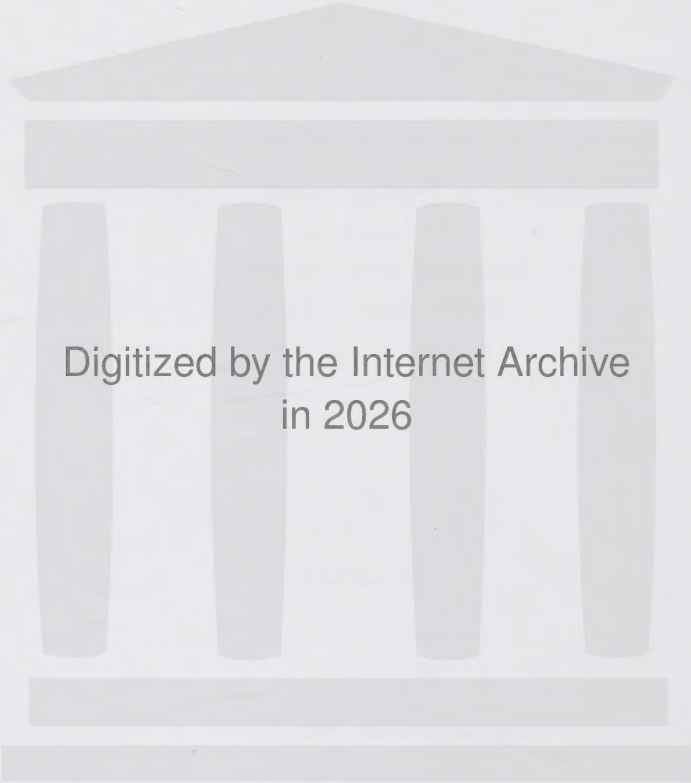
Inaugural-Dissertation

zur Erlangung der philosophischen Doktorwürde
vorgelegt der
Philosophischen Fakultät II
der Universität Zürich

von
HANSJÜRG HOTZ
von Zürich

Begutachtet von den Herren
Prof. Dr. H. Burla
und
Prof. Dr. T. Uzzell (Philadelphia)

Zürich 1983
Zentralstelle der Studentenschaft



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41507207_0096

GENIC DIVERSITY AMONG WATER FROG GENOMES INHERITED WITH AND WITHOUT RECOMBINATION

CONTENTS

1	Introduction	3
	Acknowledgments	7
2	Material and methods	8
3	Results and interpretation	15
3.1	Genic diversity in Mendelian populations and in hemiclinal lineages .	15
	Polymorphism and number of alleles	23
	Heterozygosity	28
	Clonal diversity in <u>ridibunda</u> genomes of hybridogenetic hybrid lineages	33
3.2	Sources of heterozygosity in hybrids	37
3.3	Sources of clonal diversity among hybrid lineages sympatric with <u>Rana ridibunda</u>	40
3.4	A complex pattern of clonal diversity among hybrid lineages in Italy	41
	Sources of local clonal diversity	42
	Did clonally inherited genomes evolve after clone formation?	43
	Interregional clonal variation: Implications for origin and age of hybridogenetic lineages	44
3.5	The success of hemiclinal hybrid lineages	48
4	Discussion	53
5	Summary	62
	Appendix 1. Locality list	64
	Appendix 2. Electrophoretic phenotypes and allele distributions	71
	Appendix 3. Ploidy: Evidence from erythrocyte size and electrophoresis .	103
	Appendix 4. Electrophoresis of soma and germ line of hybrids: Hybridogenesis and identification of parental genomes	111
	Appendix 5. Population systems of hybrid lineages	118
	Literature cited	123

TABLES

Table 1. List of taxa and sample sizes	10
Table 2. Sex and age classes in samples	11
Table 3. Allelic frequencies	16
Table 4. Allelic frequencies of non- <u>ridibunda</u> parental species determined from hybrid plus nonhybrid samples	21
Table 5. Polymorphism and number of alleles: Sample values	24
Table 6. Polymorphism and number of alleles: Summary	26
Table 7. Heterozygosity: Values for 20 structural gene loci among samples	29
Table 8. Heterozygosity: Sample means	31
Table 9. Heterozygosity: Summary	32
Table 10. Clones of <u>ridibunda</u> genomes in hybridogenetic hybrid lineages	34
Table 11. Clonal diversity at a site in hybridogenetic hybrid lineages	35
Table 12. Averaged genetic distances	38
Table 13. Genetic distances among <u>ridibunda</u> genomes in samples of <u>Rana</u> <u>ridibunda</u> and of hybrids	46
Table 14. Distribution and frequencies of nonhybrids and hybrids in local populations within the Italian region	49
Table 15. Summary of variation in erythrocyte size	104
Table 16. Regional differences of erythrocyte size within taxa	106
Table 17. Differences in erythrocyte size between sympatric taxa	107
Table 18. Intertaxon differences in erythrocyte size	107
Table 19. Electrophoretic comparison of soma and primary oocytes of hybrid females	112
Table 20. Electrophoretic comparison of soma and testis extracts of hybrid males	114

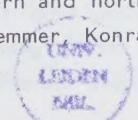
FIGURES

Fig. 1. Map of localities for the replicate water frog samples	9
Fig. 2. Phenogram of Rogers genetic distances between samples of <u>Rana</u> <u>ridibunda</u> and of <u>ridibunda</u> genomes in hybrids	47
Fig. 3. Map of Italy with localities for samples of nonhybrid and hybrid water frogs	51

1 INTRODUCTION

Among vertebrate animals, an increasing number - presently more than 50 - of naturally occurring taxa with derived clonal reproduction have been discovered, mainly in the last three decades. Taxonomically they are clustered and occur among fishes, amphibians and reptiles. All well-studied clonal vertebrates - approximately half of those presently known - have been demonstrated to be of interspecies hybrid origin. All these clusters have congeneric species with Mendelian inheritance. This low taxonomic rank of clonal vertebrate groups suggests that lack of recombination may severely reduce their evolutionary potential. Crucial questions about the evolutionary significance of recombination (for example Williams 1975, Maynard Smith 1978) include the amount and the sources of genetic variation present in such clonal taxa, their spatial and temporal adaptive success and competitive ability, and their load of deleterious mutations (Muller's ratchet mechanism: Muller 1964).

An excellent system for addressing such questions exists in western Palearctic water frogs of the family Ranidae (literature reviewed by Berger 1973 a, 1977, 1983; Hotz 1974; Dubois 1977; Hotz and Bruno 1980; see also symposium, Mitteilungen aus dem zoologischen Museum in Berlin 55 (1) : 1-229, 1979). Among this taxonomically and biogeographically complex group of semiaquatic frogs a minimum of seven Mendelian taxa, representing five to seven species, are presently distinguished: Rana ridibunda Pallas 1771 (from central Asia through much of southwestern and western Palearctic Asia to southeast, east and central Europe); Rana lessonae Camerano 1882 (from northern Italy to central, southern parts of north, and east Europe); an unnamed Italian taxon related to Rana lessonae ("Italian nonhybrid", peninsular Italy, Corsica and Sicily; Uzzell and Hotz 1979); an unnamed Yugoslavian taxon possibly related to the Rana lessonae/Italian nonhybrid stock (Adriatic southwestern Yugoslavia; Hotz and Uzzell 1982); an unnamed Greek taxon related to the Yugoslavian taxon (Adriatic northwestern Greece; Hotz and Uzzell 1982, Tunner and Heppich 1982); Rana perezi Seoane 1885 (Iberian peninsula and southern France); Rana saharica Boulenger 1913 (Maghreb region and oases of the northwestern and northern Sahara); and perhaps a second northern African species (Hemmer, Konrad and Bachmann 1980).



In addition, three different groups of geographically widespread and abundant natural interspecies hybrid lineages are known, all involving Rana ridibunda as one parental species. The other parental species is Rana lessonae in central and eastern Europe (Berger 1967, 1968), the hybrid lineages being collectively known as Rana esculenta Linnaeus 1758. In southern France, Rana perezi is the other parental species of such hybrid lineages (Graf, Karch and Moreillon 1977); and in peninsular Italy, the Italian non-hybrid (Uzzell and Hotz 1979), the hybrid lineages being referred to as "Italian hybrid".

In all three cases of interspecific hybrid lineages, diploid hybrids of both sexes reproduce via hybridogenetic (Schultz 1969) gametogenesis: only the complete genome of one parental species, Rana ridibunda, is usually clonally transmitted to progeny, the genome of the other parental species being lost in the germ line before gametogenesis is completed (Tunner 1973, 1974 for Rana esculenta; Graf, Karch and Moreillon 1977 for Rana ridibunda × Rana perezi hybrids; Uzzell and Hotz 1979 for Rana ridibunda × Italian taxon hybrids). Such hemiclonal (Kallman in Vrijenhoek, Angus and Schultz 1977) hybridogenetic reproduction is also known in fishes of the family Poeciliidae (interspecific Poeciliopsis hybrids; literature reviewed by Schultz 1977) and perhaps occurs in newts of the family Salamandridae (interspecific Triturus hybrids; see Fuhn, Sova and Dumitrescu 1975). To explain hybridogenetic gametogenesis in water frog hybrids, a premeiotic germ line exclusion of the non-ridibunda genome has been proposed, apparently induced by the ridibunda genome (Uzzell, Hotz and Berger 1980), which is followed by a regulatory endoreduplication of the ridibunda genome, after which an orderly meiosis occurs, with synapsis of sister chromatid derived chromosomes (see Graf, Karch and Moreillon 1977; Graf and Müller 1979; Uzzell, Hotz and Berger 1980; Tunner 1980; Tunner and Heppich 1981; Heppich, Tunner and Greilhuber 1982; Hotz and Uzzell 1983).

The hybridogenetic hybrids as a rule coexist with the non-ridibunda parental species in mixed populations. While matings between two hybrids in most cases give lethal progeny, somatic hybridity is restored in each generation of hybrid lineages by matings between the hybrid and its syntopic parental species: the excluded genome is thereby replaced by another, drawn anew in each generation from the gene pool of the non-ridibunda parent via sexual parasitism on its gametes. Subsequent to hybrid formation, the hybridogenetic lineages therefore persist in such hybrid/host spe-

cies populations independent from Rana ridibunda. Hybrid lineages in fact inhabit large areas outside of the present autochthonous range of Rana ridibunda in western central Europe, in southern France, and, especially remote, in Italy, where they occur throughout the Apennine peninsula and on Sicily. Whether the clonally inherited ridibunda genomes of these Italian hybrids arrived through past hybridizations between the Italian taxon and Rana ridibunda, the latter subsequently becoming extinct, whether introduction by man is involved, or whether they arrived from sympatry areas of Rana ridibunda and Rana lessonae northeast of Italy, being carried southwards by hybridogenetic Rana esculenta lineages, is not known. Biogeographical data suggest that the latter is more probable (Uzzell and Hotz 1979). Sicily has been disconnected from the mainland since the Riss II glacial period (for example Pfannenstiel 1954); although introduction by man cannot presently be ruled out, ridibunda genomes in Sicilian hybrid lineages may therefore have persisted with clonal inheritance for at least 200,000 years, the oldest age so far proposed for any clonal vertebrate.

The present study takes advantage of this wide geographical distribution and the possibly great age of the hybridogenetic hybrid lineages in Italy to examine the effects of non-recombinational reproduction on their genetic organization. Electrophoretically demonstrable minima of genic variation are determined, using proteins encoded by about 20 structural gene loci, in a survey of postlarval frog samples from natural populations on either side of the Adriatic Sea. I compare two replicate local samples each of: (1) hybridogenetic hybrids from four regions in the Italian peninsula and on Sicily, allopatric to Rana ridibunda; (2) hybridogenetic hybrids from a Balkan region where Rana ridibunda and Rana lessonae are sympatric and hybrid synthesis therefore can presently occur; and (3) Mendelian populations of Rana ridibunda from five regions of the Balkan peninsula. The comparisons include in addition: (4) the non-ridibunda parental species syntopic with the hybrids; and (5) two replicate local samples each of the Mendelian Yugoslavian and Greek taxon sympatric with Rana ridibunda. Additional water frog samples collected throughout the Italian region are used to determine the distribution and local frequencies of hybrids.

It is expected (1) that the hybridogenetic hybrids will exhibit a higher amount of individual heterozygosity than either Mendelian species, due to their interspecific hybrid genotypes (see Uzzell and Berger 1975, Uzzell and Hotz 1979); (2) that the amount of clonal diversity between the clonally

inherited ridibunda genomes of hybrids at a site will be higher within the Rana ridibunda range, where hybrid formation is possible, than outside of it; (3) if the hybrids have immigrated exclusively from the northeast into Italy, that the clonal diversity between their ridibunda genomes will decrease along a north-south gradient of increasing distance from the Rana ridibunda area; and (4) if the hybridogenetic lineages in Italy are old, that mutations will have occurred in the clonally inherited genomes. Specific questions concern (1) the amount and the patterns of genic diversity in the hemiclonally reproducing hybrid lineages compared to the Mendelian species; (2) possible identifications of the sources of clonal diversity at a site and their relative importance; (3) possible evidence for evolution in clonally inherited genomes subsequent to hybrid formation; (4) possible implications for the geographic origin and the age of hybridogenetic lineages in Italy; and (5) the amount of spatial and temporal success of the hybrid hemiclones and its probable causes.

ACKNOWLEDGMENTS

My co-chairman Prof. H. Burla steadily supported my research and liberally guided this work. He was kindly ready for help and advice each time when I needed it. My co-chairman Prof. T. Uzzell introduced me to electrophoretic technique in 1973, and the time spent since then in joint laboratory and field work, the influence of his critical thinking and the shared excitement of stimulating discussions provided a main basis for this research.

Prof. P. S. Chen and Dr. H. Jungen kindly read and commented on the manuscript. Dr. M. Bienz, Dr. J. Binkert, Dr. P. Borner, Prof. P. S. Chen, PD Dr. E. Hauschteck-Jungen, Dr. H. Jungen and D. Vismara (Zürich) and Dr. J. Szymura (Kraków) helped with advice about laboratory techniques. Dr. G. Bächli, P. Jost, Dr. H. Jungen, E. Schatzmann and many individuals from the Rechenzentrum (University of Zürich) helped with statistics and computer facilities. Dr. H. Jungen and Dr. G. Ribi offered useful suggestions on the manuscript. M.-L. Häusermann and D. Kobelt developed many films. S. Bruno (Pescasseroli), M. Frauenfelder (Zürich), M. Józefiak (Słupia Wielka) and T. Uzzell helped in field work. S. Bruno, Dr. L. Naviglio (Pescasseroli) and Dr. L. Vitelli (Pisa) collected frogs. Drs. F. and A. Schneider (Meggen) lent their car for field work. Dr. H. and R. Heusser (Maur) continuously provided warm hospitality during this work.

To each of these individuals I extend my grateful thanks.

Field work was supported by awards from the Karl Hescheler-Stiftung, Zürich.

2 MATERIAL AND METHODS

The water frogs used for the present study include 664 postlarval individuals collected during the summer of 1980, in two replicate samples each from five Balkan regions (within the present autochthonous range of Rana ridibunda) and from four Italian regions (outside of the present autochthonous range of Rana ridibunda). These 18 localities, none exceeding 200 m above sea level in altitude, are mapped in Fig. 1 and listed in Appendix 1. The taxa collected and the respective sample sizes are presented in Table 1; Table 2 lists sample sizes by sex and age classes. Although a sample of 20 individuals was planned for each local population of Rana ridibunda and of hybrids in the Italian region, fewer were obtained at Skadarsko Jezero-1 and Igoumenitsa because the samples collected included individuals of an unnamed species syntopic with Rana ridibunda that was not originally recognized in the field (see Hotz and Uzzell 1982); and at Lavello-1 because of the difficulty of distinguishing Italian nonhybrids and hybrids in the field (see Uzzell and Hotz 1979).

In addition, 689 postlarval frogs in 70 samples from the Italian region, including 61 other localities, collected between 1974 and 1982, are used to evaluate the success of hybridogenetic hybrid lineages by determining their distribution and their relative frequencies in local populations. For these additional samples, nonhybrids and hybrids were discriminated using electrophoretic phenotypes for at least three diagnostic enzyme loci (Uzzell and Hotz 1979); this electrophoresis was carried out for the 1974 samples by T. Uzzell, for the others, by T. Uzzell and myself. These additional localities are listed in Appendix 1 (see Fig. 3, p. 51).

Each frog was heparinized by injecting 0.1-0.5 ml of a Na-heparin (~20 mg/ml in 0.7 % NaCl) solution into the abdominal cavity, anesthetized with ether, and bled from the aorta. A sample of blood was streaked on a glass slide and air-dried for erythrocyte examination. After centrifugation at 600 g for 4 min, the plasma was frozen at -22°C. Heart, liver, kidneys, gonads and the remaining carcasses were separately frozen at -22°C.

The proteins used, encoded by 20 structural gene loci, include the enzymes aspartate aminotransferase (AAT, Enzyme Commission 2.6.1.1), ade-

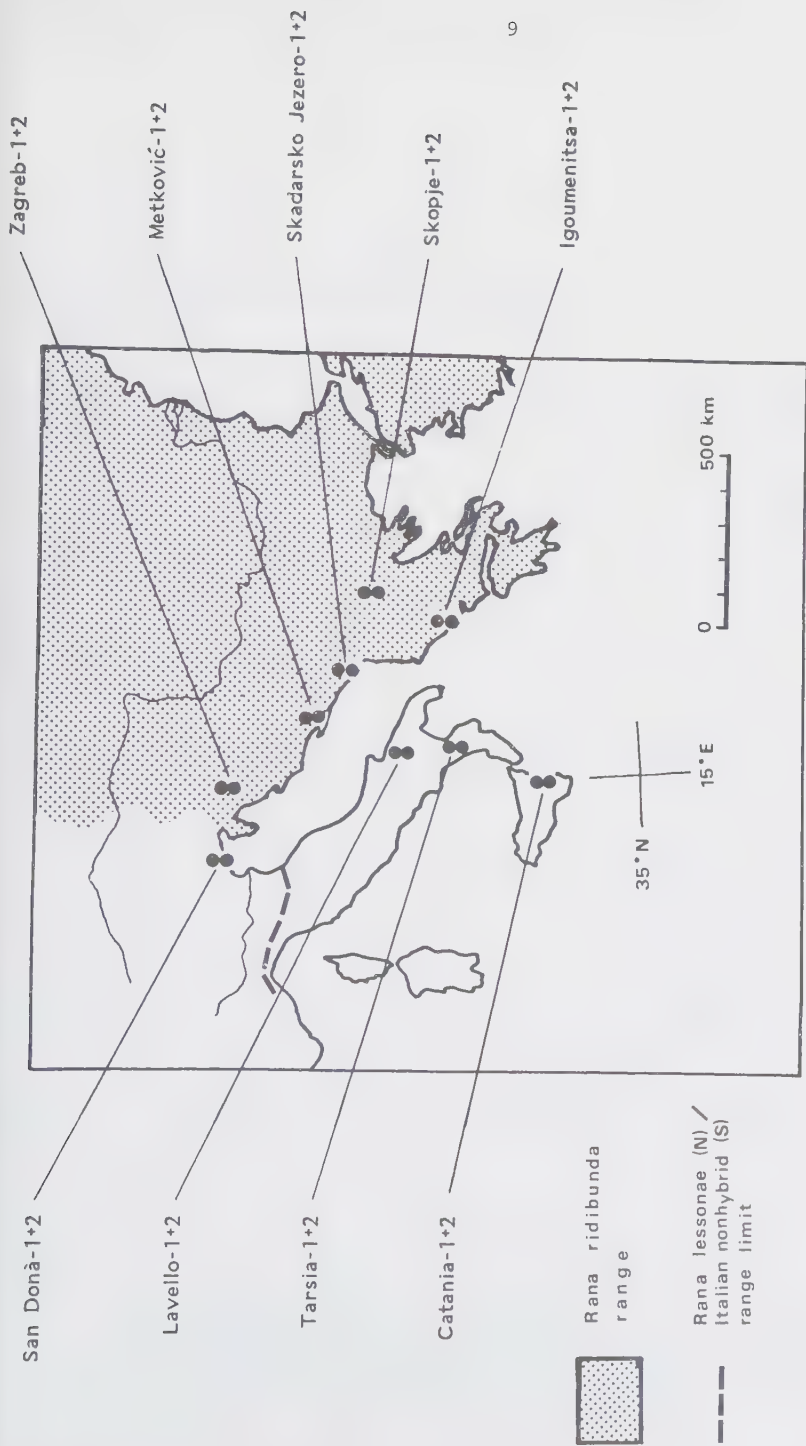


Fig. 1. Map of the central northern Mediterranean area showing collecting localities (listed in Appendix 1) for the replicate water frog samples discussed. The autochthonous present range of *Rana ridibunda* and the approximate position of the range limits between *Rana lessonae* and the Italian nonhybrid are indicated.

Table 1. List of taxa and sample sizes of the replicate water frog samples discussed. Taxa were identified by electrophoresis. Hybrids and backcrosses between *Rana ridibunda* and either Balkan taxon (5 from Skadarsko Jezero-1, 1 from Igoumenitsa-1, 2 from Igoumenitsa-2) are omitted and have been discussed elsewhere (Hotz and Uzzell 1982). Localities are listed in Appendix 1 and mapped in Fig. 1.

Locality	Mendelian populations				Hybridogenetic hybrids		Total of individuals
	Rana ridibunda	Non-ridibunda parental species	Unnamed Balkan species		Rana esculenta	Italian hybrid	
		Rana lessonae	Italian nonhybrid	Yugoslavian taxon			
Balkan region:							
Zagreb-1	21				17		38
Zagreb-2	21	1			17		39
Metković-1	26						26
Metković-2	26						26
Skadarsko Jezero-1	6			31			37
Skadarsko Jezero-2	20			9			29
Skopje-1	25						25
Skopje-2	41						41
Igoumenitsa-1	5				27		32
Igoumenitsa-2	18				11		29
Italian region:							
San Donà-1		1			30		31
San Donà-2		1			42		43
Lavello-1			34			11	45
Lavello-2			3			36	39
Tarsia-1			2			33	35
Tarsia-2						29	29
Catania-1			23			58	81
Catania-2						31	31
Total	209	3	62	40	106	198	656

nylate kinase (AK, EC 2.7.4.3), creatine kinase (CK, EC 2.7.3.2), α -glycerophosphate dehydrogenase (α -GDH, EC 1.1.1.8), glucosylphosphate isomerase (GPI, EC 5.3.1.9), 2 loci of NADP-dependent isocitrate dehydrogenase (IDH, EC 1.1.1.42), 2 loci of lactate dehydrogenase (LDH, EC 1.1.1.27), 2 loci of NAD-dependent malate dehydrogenase (MDH, EC 1.1.1.37), mannosephosphate isomerase (MPI, EC 5.3.1.8), a peptidase cleaving l-leucyl-l-tyrosine and l-leucyl-l-valine (Pep, EC 3.4), phosphoglucomutase (PGM, EC 2.7.5.1), 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.43), and superoxide dismutase (SOD, EC 1.15.1.1), as well as albumin (Alb) and 3 loci of soluble muscle proteins (MProt). In addition, the enzymes aldolase (Ald, EC 4.1.2.13) and glutamate dehydrogenase (GDH, EC 1.4.1.2) are occasionally referred to.

For enzyme electrophoresis of somatic tissues, small pieces of frozen skeletal or heart muscle, liver or kidney were crushed in equal volumes of distilled water; liver and kidney extracts were subsequently centrifuged at 1800 g for 15 min at 4°C. For enzyme electrophoresis of gonadal tissues, pieces of frozen testes were crushed in equal volumes of distilled water; individual enlarged primary oocytes of average size from frozen ovaries, after drying the surface on filter paper, were squashed on small filter paper tabs (usually 1, sometimes 2 to several per tab) to which 3-10 μ l of distilled water were added.

The extracts were applied on filter paper tabs (Whatman no. 3) to 12 % or 15 % starch gels (Electrostarch lot 307, or an approximately 1:1 mixture of Electrostarch lot 392 and Sigma starch lot 11F-0477). Two continuous tris-citrate buffer systems were mainly used: one with low pH, primarily for AAT, AK, CK, α -GDH, GPI, LDH, MDH, MPI, PGM and SOD (pH 5.7-5.9; electrode buffer 0.22 M trishydroxymethylaminomethane + 0.09 M citric acid, gel buffer 0.008 M tris + 0.003 M citric acid), and one with high pH, primarily for Ald, GDH, IDH, LDH, Pep, 6PGDH and SOD (pH 8.0; electrode buffer 0.69 M tris + 0.14 M citric acid, gel buffer 0.023 M tris + 0.005 M citric acid). For verification of LDH phenotypes, a discontinuous Poulik buffer (Yang 1971) was also used (electrode buffer 0.30 M boric acid + 0.06 M NaOH, pH 8.2; gel buffer 0.076 M tris + 0.005 M citric acid, pH 8.7). Starch gels were heated to 83°C and then degassed. For some NADP-dependent enzymes (mainly 6PGDH, IDH, GDH and MPI), 2-4 drops/100 ml gel buffer of 0.04 M Na- β -NADP solution were usually added, immediately before degassing. Proteins were separated horizontally at 10 or 13 V/cm for 3-4 h

(low pH tris-citrate gels), at 9 V/cm for 4-6 h (high pH tris-citrate gels), and at 14 or 17 V/cm for 3-5 h (Poulik gels) in a refrigerator at approximately 4°C. Gels were covered with a plastic foil, with or without pans of crushed ice on them. The filter paper tabs were removed after the first 15 min of electrophoresis.

Enzymes were localized on 1.5 mm thick gel slices with modifications of standard chromogenic methods, such as those reported by Shaw and Prasad (1970), Yang (1971), and Harris and Hopkinson (1976). All but AAT were developed in 0.5-1 % agar overlays rather than in solutions.

For electrophoresis of plasma and muscle proteins for non-specific staining, 0.5-4 μ l samples of plasma (undiluted or diluted with 0.7 % NaCl solution) and of aqueous skeletal muscle extracts (obtained by sonicating small pieces in 2-3 volumes of water) were used. They were placed in 6 or in 9 mm wide pockets in 1 mm thick discontinuous polyacrylamide gels (stacking gel 2.5 % acrylamide + 0.6 % N,N'-methylene-bisacrylamide, separating gel 7.5 % acrylamide + 0.2 % bisacrylamide, electrode buffer 0.005 M tris + 0.038 M glycine, pH 8.4). Proteins were separated vertically at 14-18 V/cm for 4-6 h, without cooling. They were stained for 30 min (plasma) or 50 min (muscle extracts) in 1 % Coomassie Blue Fast R dissolved in methanol, water and glacial acetic acid (5:5:1), and destained for one half to two days in the same solution without Coomassie Blue. These polyacrylamide gels were also used for electrophoresis of plasma LDH.

For each locus studied, all electrophoretic phenotypes observed for any taxon from each region were calibrated by using samples of control individuals on the same gels, or by applying tissue extracts of the individuals to be compared adjacent to each other on single gels; for some phenotypes with similar mobilities, comparisons were made by repeatedly alternating them. Heterozygotes for rare alleles were also placed adjacent to each other on single gels. The stained gel slices were photographed.

For multilocus enzyme systems (AAT, IDH, LDH, MDH, Pep, PGM) numbers are assigned to loci in sequence of decreasing anodal mobility of the allelic product most common in Rana ridibunda. Allelic variants at a locus are named by assigning them letters alphabetically (see Hotz and Uzzell 1982). Assumptions used for genetic interpretation of the observed electrophoretic phenotypes are listed in Appendix 2.

Genetic distances were calculated from the observed allelic frequencies for 19 or 17 structural gene loci, using the measures proposed by Nei (1972, 1973; standard distance) and Rogers (1972). The genetic distances obtained were used to construct phenograms (complete linkage cluster analysis; Sneath and Sokal 1973).

The sizes of 10 air-dried erythrocytes per individual were measured by marking the extreme length and width in a camera lucida view (magnification 600-800 $\times$). These marked lengths and widths were then measured to the nearest tenth of a millimeter with a ruler. Cross-sectional areas of the ellipsoid erythrocytes were estimated as $\pi \times \text{length} \times \text{width}/4$. The significance of differences between sexes, age classes, localities, and electrophoretically defined taxa was tested using a Mann-Whitney U test and a Kruskal-Wallis H test (BMD P3S; see Dixon Ed. 1981).

3 RESULTS AND INTERPRETATION

Results that are indirectly pertinent to the main questions of this study are presented in Appendices 2 through 5. Appendix 2 contains, for each locus studied, a detailed description of the electrophoretic phenotypes observed, their genetic interpretation, the taxonomic and geographic distribution of the electrophoretic alleles among the samples discussed and, when known, among other western Palearctic water frogs; Appendix 3 provides estimates of ploidy of all individuals by cell size measurements and electrophoretic data; in Appendix 4 electrophoretic comparison between somatic tissues and germ line cells of hybrids is used to demonstrate hybridogenesis and to assign alleles of hybrids to parental genomes; and Appendix 5 provides background knowledge, assumed throughout this paper, about population structure and mode of reproduction in the hybrid lineages discussed.

3.1 GENIC DIVERSITY IN MENDELIAN POPULATIONS AND IN HEMICLONAL LINEAGES

The electrophoretically detected genetic variation in the water frog samples discussed is summarized as allele frequencies for 20 structural gene loci in Table 3. Since the small to zero sample sizes of the non-ridibunda parental species of hybrids at Zagreb and at 6 of the 8 Italian localities preclude a representative calculation of allele frequencies, I redetermined these nonhybrid allele frequencies using pooled samples of non-ridibunda genomes in nonhybrids and in hybrids (Table 4). This is justified because the non-ridibunda genomes in hybridogenetic hybrid lineages are drawn anew in each generation from the gene pool of the syntopic non-ridibunda parent (Appendix 5); to take these values as representative for the local nonhybrid populations assumes, however, that all non-ridibunda genomes combine equally well with the ridibunda genomes of hybrids.

Table 3. Allelic frequencies. Electrophoretically detected allele frequencies for 20 structural gene loci. Localities are listed in Appendix 1 and mapped in fig. 1. Taxa were identified by electrophoresis. Hybrids and backcrosses between *Rana ridibunda* and either Balkan taxon are omitted (see Hotz and Uzzell 1982). Two alleles were counted for each of two possible triploids (Appendix 3). Frequencies at a locus do not always sum to 1.00 because of rounding. N = number of individuals. - = allele absent; for 3 loci (MPI, GPGH, Alb): * = allele present, blank = not scored or presence or absence of allele uncertain (Appendix 2). For MPI phenotypes at Catania see Appendix 2.

Taxon	Locality	N	AAT-1										AK		CK		a-GDH					
			a	b	c	d	e	f	g	h	i	j	a		a	b	a	b	c	d		
<i>Rana ridibunda</i>																						
	Zagreb-1	21	-	-	-	-	1.00	-	-	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	Zagreb-2	21	-	-	.02	-	.98	-	-	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	Metkovic-1	26	-	-	.06	-	.94	-	-	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	Metkovic-2	26	-	.02	.04	-	.94	-	-	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	Skadarsko Jezero-1	6	-	-	-	1.00	-	-	-	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	Skadarsko Jezero-2	20	-	-	-	1.00	-	-	-	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	Skopje-1	25	-	-	.02	-	.98	-	-	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	Skopje-2	41	-	-	.04	-	.94	.02	-	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	Igoumenitsa-1	5	-	-	-	1.00	-	-	-	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	Igoumenitsa-2	18	-	-	-	-	.86	.14	-	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
<i>Rana lessonae</i>																						
	Zagreb-2	1	-	-	-	-	-	-	1.00	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	San Donà-1	1	-	-	-	-	-	-	1.00	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
	San Donà-2	1	-	-	-	-	-	-	1.00	-	-	-	1.00	-	-	1.00	1.00	-	1.00	-	-	-
<i>Italian nonhybrid</i>																						
	Lavello-1	34	.15	-	.04	-	.81	-	-	-	-	-	1.00	.82	.18	-	1.00	-	1.00	-	-	-
	Lavello-2	3	.33	-	-	-	.67	-	-	-	-	-	1.00	.83	.17	-	1.00	-	1.00	-	-	-
	Tarsia-1	2	-	-	.50	-	.50	-	-	-	-	-	1.00	1.00	-	-	1.00	-	1.00	-	-	-
	Catania-1	23	-	-	-	-	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-	.98	-	.02	-
<i>Yugoslavian taxon</i>																						
	Skadarsko Jezero-1	31	-	-	-	-	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
	Skadarsko Jezero-2	9	-	-	-	-	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-
<i>Greek taxon</i>																						
	Igoumenitsa-1	27	-	-	-	-	.98	.02	-	-	-	-	1.00	-	1.00	-	1.00	-	.94	.06	-	-
	Igoumenitsa-2	11	-	-	-	-	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-	.96	.05	-	-
<i>Rana esculenta</i>																						
	Zagreb-1	17	-	-	-	-	.50	-	.50	-	-	-	1.00	-	1.00	1.00	.50	.50	.50	-	-	-
	Zagreb-2	17	-	-	-	-	.50	-	.50	-	-	-	1.00	-	1.00	1.00	.50	.50	.50	-	-	-
	San Donà-1	30	-	-	-	-	.52	-	.47	-	.02	-	1.00	-	1.00	1.00	.50	.50	.50	-	-	-
	San Donà-2	42	-	-	-	-	.52	-	.48	-	-	-	1.00	.01	.99	.50	.49	.50	.50	.01	-	-
<i>Italian hybrid</i>																						
	Lavello-1	11	-	-	-	-	.77	-	-	-	-	.23	1.00	.46	.55	.50	.50	.50	.50	-	-	-
	Lavello-2	36	.03	-	.01	-	.86	-	-	-	.10	-	1.00	.40	.60	.50	.50	.50	.50	-	-	-
	Tarsia-1	33	-	-	.15	.83	-	-	.02	-	-	-	1.00	.35	.65	.50	.50	.50	.50	-	-	-
	Tarsia-2	29	-	.05	.09	.86	-	-	-	-	-	-	1.00	.45	.55	.50	.50	.50	.50	-	-	-
	Catania-1	58	-	-	-	-	1.00	-	-	-	-	-	1.00	-	1.00	1.00	.50	.50	.50	-	-	-
	Catania-2	31	-	-	-	-	1.00	-	-	-	-	-	1.00	-	1.00	1.00	.50	.50	.50	-	-	-

Table 3 (continued).

Taxon	Locality	N	GPI							IDH-1			IDH-2		LDH-1							LDH-2	
			a	b	c	d	e	f	g	a	b	c	a	b	a	b	c	d	e	f	a	b	
Rana ridibunda	Zagreb-1	21	.36	-	-	.64	-	-	-	1.00	-	-	1.00	.60	-	.40	-	-	-	-	.95	.05	
	Zagreb-2	21	.43	-	-	.57	-	-	-	1.00	-	-	1.00	.52	-	.48	-	-	-	-	.93	.07	
	Metkovic-1	26	.63	-	-	.37	-	-	-	1.00	-	-	1.00	1.00	1.00	-	-	-	-	-	1.00	-	
	Metkovic-2	26	.48	-	-	.52	-	-	-	1.00	-	-	1.00	1.00	1.00	-	-	-	-	-	1.00	-	
	Skadarsko Jezero-1	6	.83	-	.08	.52	-	-	-	1.00	-	-	1.00	1.00	1.00	-	-	-	-	-	1.00	-	
	Skadarsko Jezero-2	20	.70	-	.03	.18	.10	-	-	1.00	-	-	1.00	1.00	1.00	-	-	-	-	-	1.00	-	
	Skopje-1	25	.38	-	-	.60	.02	-	-	1.00	-	-	1.00	1.00	1.00	-	-	-	-	.98	.02		
	Skopje-2	41	.43	.01	-	.55	.01	-	-	1.00	-	-	1.00	.99	1.00	.01	-	-	-	-	1.00	-	
	Igoumenitsa-1	5	.40	-	-	.60	-	-	-	1.00	-	-	1.00	1.00	1.00	-	-	-	-	-	1.00	-	
	Igoumenitsa-2	18	.36	.08	-	.56	-	-	-	1.00	-	-	1.00	1.00	1.00	-	-	-	-	-	1.00	-	
Rana lessonae	Zagreb-2	1	-	-	-	1.00	-	-	-	1.00	-	-	1.00	-	1.00	-	-	-	-	-	1.00	-	
	San Donà-1	1	-	-	-	1.00	-	-	-	1.00	-	-	1.00	-	1.00	-	-	-	1.00	-	1.00	-	
	San Donà-2	1	-	-	-	1.00	-	-	-	1.00	-	-	1.00	-	1.00	-	-	-	1.00	-	1.00	-	
Italian nonhybrid	Lavello-1	34	-	-	-	1.00	-	-	-	.93	.07	-	1.00	-	-	-	-	-	-	-	1.00	-	
	Lavello-2	3	-	-	-	1.00	-	-	-	.83	.17	-	1.00	-	-	-	-	-	-	-	1.00	-	
	Tarsia-1	2	-	-	-	1.00	-	-	-	1.00	-	-	1.00	-	-	-	-	-	-	-	1.00	-	
	Catania-1	23	-	-	-	1.00	-	-	-	1.00	-	-	1.00	-	-	-	-	-	-	-	1.00	-	
	Catania-2	23	-	-	-	1.00	-	-	-	1.00	-	-	1.00	-	-	-	-	-	-	-	1.00	-	
Yugoslavian taxon	Skadarsko Jezero-1	31	-	-	-	1.00	-	-	-	1.00	-	-	1.00	-	-	1.00	-	-	-	-	1.00	-	
	Skadarsko Jezero-2	9	-	-	-	1.00	-	-	-	1.00	-	-	1.00	-	-	1.00	-	-	-	-	1.00	-	
Greek taxon	Igoumenitsa-1	27	-	-	-	1.00	-	-	-	1.00	-	-	1.00	-	-	1.00	-	-	-	-	1.00	-	
	Igoumenitsa-2	11	-	-	-	1.00	-	-	-	1.00	-	-	1.00	-	-	1.00	-	-	-	-	1.00	-	
Rana esculenta	Zagreb-1	17	.21	-	-	.76	.03	-	-	1.00	-	-	1.00	-	-	.50	-	-	.35	-	1.00	-	
	Zagreb-2	17	.15	-	-	.76	.09	-	-	.03	.97	-	1.00	-	-	.26	.50	-	.24	-	1.00	-	
	San Donà-1	30	.50	-	-	.48	.01	-	.02	1.00	-	-	1.00	-	-	.18	.50	-	.32	-	1.00	-	
	San Donà-2	42	.51	-	-	.48	.01	-	-	.99	.01	-	.01	.99	-	.25	.50	-	.25	-	1.00	-	
	Italian hybrid	11	.50	-	-	.50	-	-	-	.95	.05	-	1.00	-	-	.50	-	-	.45	.05	1.00	-	
Italian hybrid	Lavello-1	36	.20	-	-	.50	-	-	-	.94	.06	-	1.00	-	-	.50	-	-	.50	-	1.00	-	
	Lavello-2	33	.23	-	-	.50	-	-	-	1.00	-	-	1.00	-	-	.50	-	-	.45	.05	1.00	-	
	Tarsia-1	29	.57	-	-	.45	.02	-	-	.02	.98	-	1.00	-	-	.50	-	-	.43	.07	1.00	-	
	Tarsia-2	29	.57	-	-	.45	.02	-	-	.02	.98	-	1.00	-	-	.50	-	-	.43	.07	1.00	-	
	Catania-1	58	.53	-	-	.47	-	-	-	1.00	-	-	1.00	-	-	.50	-	-	.50	-	1.00	-	
	Catania-2	31	.50	-	-	.50	-	-	-	1.00	-	-	1.00	-	-	.50	-	-	.50	-	1.00	-	

Table 3 (continued).

Taxon Locality	N	MDH-1			MDH-2			MPI										Pep-2			
		a	b	c	a	b	c	a	b	c	d	e	f	g	h	i		a	b	c	d
Rana ridibunda																					
Zagreb-1	21	-	1.00	-	1.00	.57	-	.43	-	-	-	-	-	-	-	-	-	.10	.90	-	-
Zagreb-2	21	-	1.00	-	1.00	.36	-	.64	-	-	-	-	-	-	-	-	-	.14	.86	-	-
Metković-1	26	-	1.00	-	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-
Metković-2	26	-	1.00	-	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-
Skadarsko Jezero-1	6	-	1.00	-	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-
Skadarsko Jezero-2	20	-	1.00	-	1.00	.95	.03	.03	-	-	-	-	-	-	-	-	-	-	1.00	-	-
Skopje-1	25	-	1.00	-	1.00	.84	.04	.12	-	-	-	-	-	-	-	-	-	.05	.95	-	-
Skopje-2	41	-	1.00	-	1.00	.77	-	.23	-	-	-	-	-	-	-	-	-	-	.95	-	-
Igoumenitsa-1	5	-	1.00	-	1.00	.70	.30	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-
Igoumenitsa-2	18	-	1.00	-	1.00	.69	.31	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-
Rana lessonae																					
Zagreb-2	1	-	1.00	-	1.00	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	1.00	-
San Donà-1	1	-	1.00	-	1.00	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	1.00	-
San Donà-2	1	-	1.00	-	1.00	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	1.00	-
Italian nonhybrid																					
Lavello-1	34	-	1.00	-	1.00	-	-	-	-	-	-	-	-	*	*	*	-	-	-	1.00	-
Lavello-2	3	-	1.00	-	1.00	-	-	-	-	-	-	-	-	-	-	1.00	-	-	-	1.00	-
Tarsia-1	2	-	.75	.25	1.00	-	-	-	-	*	*	-	*	-	-	*	-	-	-	1.00	-
Catania-1	23	-	-	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.89	.11
Yugoslavian taxon																					
Skadarsko Jezero-1	31	-	1.00	-	1.00	-	-	-	.97	.03	-	-	-	-	-	-	-	-	-	1.00	-
Skadarsko Jezero-2	9	-	1.00	-	1.00	-	-	-	.94	.06	-	-	-	-	-	-	-	-	-	1.00	-
Greek taxon																					
Igoumenitsa-1	27	1.00	-	-	1.00	-	.85	.15	-	-	-	-	-	-	-	-	-	.05	.95	-	-
Igoumenitsa-2	11	1.00	-	-	1.00	-	.91	.09	-	-	-	-	-	-	-	-	-	-	-	-	-
Rana esculenta																					
Zagreb-1	17	-	1.00	-	1.00	.06	-	.44	-	-	-	-	-	.50	-	-	-	-	.53	.47	-
Zagreb-2	17	-	1.00	-	1.00	-	-	.50	-	-	-	-	-	.50	-	-	-	-	.50	.50	-
San Donà-1	30	-	.98	.02	1.00	-	-	-	-	-	-	-	-	*	*	*	-	-	.50	.50	-
San Donà-2	42	-	1.00	-	1.00	-	-	.50	-	-	-	-	-	*	*	.01	-	-	.51	.49	-
Italian hybrid																					
Lavello-1	11	-	1.00	-	1.00	-	-	.50	-	-	-	-	-	.05	.45	-	-	-	.50	.50	-
Lavello-2	36	-	1.00	-	1.00	-	-	.50	-	-	-	-	-	*	*	*	-	-	.50	.50	-
Tarsia-1	33	-	.88	.12	1.00	-	-	.50	-	-	-	-	*	*	*	-	-	-	.50	.50	-
Tarsia-2	29	-	.86	.14	1.00	-	-	.50	-	-	-	-	*	*	*	-	-	-	.50	.50	-
Catania-1	58	-	.50	.50	1.00	-	-	.50	-	-	-	-	(.50)	-	-	-	-	-	.50	.41	.09
Catania-2	31	-	.50	.50	1.00	-	-	.50	-	-	-	-	(.50)	-	-	-	-	-	.50	.50	-

Table 3 (continued).

Taxon Locality	N	PGM-2							6PGDH					SOD		MProt1		MProt2		Mprot3
		b	c	d	e	f	g	a	b	c	d	e	a	b	c	a	b			
Rana ridibunda																				
Zagreb-1	21	.21	-	.79	-	-	-	-	.02	.69	.29		1.00	-	1.00	-	1.00	-	1.00	
Zagreb-2	21	.07	-	.93	-	-	-	-	-	.55	.45		1.00	-	1.00	-	1.00	-	1.00	
Metkovic-1	26	.02	-	.96	-	-	-.02	-	-	1.00			1.00	-	1.00	-	1.00	-	1.00	
Metkovic-2	26	.42	-	.98	-	-	-	-	-	1.00			1.00	-	1.00	-	1.00	-	1.00	
Skadarsko Jezero-1	6	.02	-	.58	-	-	-	-	-	1.00			.92	.08	1.00	-	1.00	-	1.00	
Skadarsko Jezero-2	20	.43	-	.58	-	-	-	-	-	1.00			1.00	-	1.00	-	1.00	-	1.00	
Skoplje-1	25	.14	-	.86	-	-	-	-	-.04	.92	.04		1.00	-	1.00	-	1.00	-	1.00	
Skoplje-2	41	.15	-	.85	-	-	-	-	-.02	.93	.05		1.00	-	1.00	-	1.00	-	1.00	
Igoumenitsa-1	5	-	-	1.00	-	-	-	-	-	1.00			1.00	-	1.00	-	1.00	-	1.00	
Igoumenitsa-2	18	.28	-	.72	-	-	-	-	.03	.97	-		1.00	-	1.00	-	1.00	-	1.00	
Rana lessonae																				
Zagreb-2	1	-	1.00	-	-	-	-	-	-	-	-		1.00	-	1.00	-	1.00	-	1.00	
San Donà-1	1	-	1.00	-	-	-	-	-	-	-	-		1.00	-	1.00	-	1.00	-	1.00	
San Donà-2	1	-	1.00	-	-	-	-	-	-	-	-		1.00	-	1.00	-	1.00	-	1.00	
Italian nonhybrid																				
Lavello-1	34	-	1.00	-	-	-	-	-	-	-	-		1.00	-	.01	.99	1.00	-	1.00	
Lavello-2	3	-	1.00	-	-	-	-	-	-	-	-		1.00	-	-	1.00	1.00	-	1.00	
Tarsia-1	2	-	1.00	-	-	-	-	-	-	-	-		1.00	-	-	1.00	1.00	-	1.00	
Catania-1	23	-	1.00	-	-	-	-	-	1.00	-	-		1.00	-	-	1.00	1.00	-	1.00	
Yugoslavian taxon																				
Skadarsko Jezero-1	31	1.00	-	-	-	-	-	-	-	-	-		1.00	-	1.00	-	1.00	-	1.00	
Skadarsko Jezero-2	9	1.00	-	-	-	-	-	-	-	-	-		1.00	-	1.00	-	1.00	-	1.00	
Greek taxon																				
Igoumenitsa-1	27	-	-	1.00	-	-	-	-	-	-	-		1.00	-	-	1.00	1.00	-	1.00	
Igoumenitsa-2	11	-	-	1.00	-	-	-	-	-	-	-		1.00	-	-	1.00	1.00	-	1.00	
Rana esculenta																				
Zagreb-1	17	.21	.50	.29	-	-	-	-	-	.06	.44	.44	1.00	-	1.00	-	1.00	-	1.00	
Zagreb-2	17	.06	.47	.47	-	-	-	-	-	.32	.18	.18	1.00	-	1.00	-	1.00	-	1.00	
San Donà-1	30	.50	.48	-	.02	-	-	-	-	.17	.33	.33	1.00	-	1.00	-	1.00	-	1.00	
San Donà-2	42	.50	.49	-	.01	-	-	-	-	.01	.49	.49	1.00	-	1.00	-	1.00	-	1.00	
Italian hybrid																				
Lavello-1	11	.50	.41	-	-	.09	-	-	-	.23	.27	.27	1.00	-	.50	.50	1.00	-	1.00	
Lavello-2	36	.50	.50	-	-	-	-	-	-	.39	.11	.11	1.00	-	.50	.50	1.00	-	1.00	
Tarsia-1	33	.50	.50	-	-	-	-	-	-	.09	.50	.50	1.00	-	.50	.50	1.00	-	1.00	
Tarsia-2	29	.50	.50	-	-	-	-	-	-	.21	.50	.50	1.00	-	.50	.50	1.00	-	1.00	
Catania-1	58	-	.49	.50	-	.01	-	.01	.49	.50	.50	.50	1.00	-	.50	.50	1.00	-	1.00	
Catania-2	31	-	.50	.50	-	-	-	-	-	.50	.50	.50	1.00	-	.50	.50	1.00	-	1.00	

Table 3 (continued).

Taxon	Locality	N	Alb									
			a	b	c	d	e	f	g	h	i	
Rana ridibunda	Zagreb-1	21	-	*	*	-	-	-	-	-	-	
	Zagreb-2	21	-	*	*	-	-	-	-	-	*	
	Metković-1	26	-	*	*	-	-	-	-	-	*	
	Metković-2	26	-	*	*	-	-	-	-	-	*	
	Skadarsko Jezero-1	6	-	*	*	-	-	-	-	-	-	
	Skadarsko Jezero-2	20	-	*	*	*	*	-	-	-	-	
	Skopje-1	25	-	*	*	*	*	-	-	-	-	
	Skopje-2	41	-	*	*	*	*	-	-	-	-	
	Igoumenitsa-1	5	-	*	*	*	*	-	-	-	-	
Igoumenitsa-2	18	-	*	*	*	*	-	-	-	-		
Rana lessonae	Zagreb-2	1	1.00	-	-	-	-	-	-	-	-	
	San Dona-1	1	1.00	-	-	-	-	-	-	-	-	
	San Dona-2	1	1.00	-	-	-	-	-	-	-	-	
Italian nonhybrid	Lavello-1	34	.93	-	-	-	-	-	.07	-	-	
	Lavello-2	3	.83	-	-	-	-	-	.17	-	-	
	Tarsia-1	2	.25	-	-	-	-	-	.75	-	-	
	Tarsia-2	23	.74	-	-	-	-	.26	-	-	-	
	Catania-1	23	.74	-	-	-	-	.26	-	-	-	
Yugoslavian taxon	Skadarsko Jezero-1	31	-	*	*	-	-	-	-	-	-	
	Skadarsko Jezero-2	9	-	*	*	-	-	-	-	-	-	
Greek taxon	Igoumenitsa-1	27	-	-	-	-	1.00	-	-	-	-	
	Igoumenitsa-2	11	-	-	-	-	1.00	-	-	-	-	
Rana esculenta	Zagreb-1	17	.50	.26	.24	-	-	-	-	-	-	
	Zagreb-2	17	.50	.29	.15	-	-	-	-	.06	-	
	San Dona-1	30	.50	-	-	-	-	-	-	.50	-	
	San Dona-2	42	.46	-	-	-	-	.01	.02	.50	-	
	Lavello-1	11	.36	-	-	-	-	.05	.09	-	.50	
Italian hybrid	Lavello-2	36	.39	-	-	-	-	.01	.10	-	.50	
	Tarsia-1	33	.26	-	-	-	-	.03	.21	-	.50	
	Tarsia-2	29	.21	-	-	-	-	.05	.24	-	.50	
	Catania-1	58	.28	-	-	-	-	.22	-	-	.50	
	Catania-2	31	.50	-	-	-	-	-	-	-	.50	

Polymorphism and number of alleles

The overall amount of genic variability present in a Mendelian population as well as in a local group of hemiclinal hybrid lineages is reflected in the proportion of loci that are polymorphic and in the average number of alleles per locus. These diversity estimates are presented for the samples discussed in Table 5, and summarized over all samples of the taxa compared in Table 6; Tables 3 and 4 indicate which loci and which alleles are involved.

Among the 5 Mendelian water frog taxa discussed, the percentage of 17 loci that are polymorphic (P, counting only loci at which the frequency of the most common allele was $\leq 95\%$; Table 6) was highest in Rana ridibunda, followed in decreasing sequence by the Italian nonhybrid, Rana lessonae and the Greek taxon, and was lowest in the Yugoslavian taxon (all loci monomorphic; P increases to 6% for Skadarsko Jezero-2 when MPI is included). Among samples (Table 5), polymorphism of Rana ridibunda was highest in the region of Zagreb, and decreased in sequence at Skadarsko Jezero and Skopje, Metković and Igoumenitsa; when using 19 loci (by adding the MPI and 6PGDH data), Skopje-1 (P = 21%) and Skopje-2 (26%) follow Zagreb (37% at both sites). Among the Italian nonhybrids, P was higher for the mainland Tarsia and Lavello samples than for the island samples from Catania. If polymorphism is calculated including all loci that are heteroallelic (Wright 1978), independent of allele frequencies (P'), Rana lessonae had the highest values, followed by the Italian nonhybrid and Rana ridibunda; the Greek and the Yugoslavian taxon again were least variable (Table 6). The shifting of positions between Rana ridibunda and the Rana lessonae/Italian nonhybrid stock reflects the many rare alleles that occur in the Italian nonhybrid (AAT-1 d, h, α -GDH d, GPI a, f, LDH-1 f, PGM-2 f, MProt1 b; Table 4); and it especially reflects the San Donà samples of Rana lessonae genomes that contain a huge number of rare alleles (AAT-1 e, i, CK a, α -GDH c, GPI a, f, g, IDH-1 c, MDH-1 c, Pep-2 b, PGM-2 e, and 3 additional alleles not included for the calculation: MPI i, Alb f and g; Tables 3,4); the IDH-2 a allele found in a Rana esculenta from San Donà-2, omitted because it is not assignable to a parental genome (Appendix 4), may well be a lessonae allele also. These alleles account for the extreme difference between P (6%) and P' (47%) at San Donà-2 (Table 5). Since several of these rare alleles at San Donà are otherwise characteristic for the Italian nonhybrid (CK a, IDH-1 c, MDH-1 c, MPI i, Alb f and g; AAT-1 e also is its most common allele; Table 4 and Appendix 2), it seems plausible that

Table 5. Polymorphism and number of alleles : Sample values.
The estimates are based on electrophoretic data using 17 structural gene loci (MPI, 6PGDH and Alb are omitted; Appendix 2).

P = proportion of polymorphic loci (frequency of most common allele ≤ 0.95)

P' = proportion of heteroallelic loci (independent of allele frequencies)

A = mean number of alleles per locus

A' = mean number of alleles per heteroallelic locus

NG = sample size of nonhybrids; NH = sample size of hybrids; NG = number of genomes sampled

** = calculated from pooled samples of nonhybrids and nonhybrid genomes in hybrids.

Localities are listed in Appendix 1 and mapped in Fig. 1.

Taxon	Locality	NN	NH	NG	Polymorphism		Number of alleles	
					P	P'	A	A'
(1) Genomes of Mendelian species:								
<i>Rana ridibunda</i>								
	Zagreb-1	21		42	.29	.29	1.29	2.00
	Zagreb-2	21		42	.29	.35	1.35	2.00
	Metkovic-1	26		52	.12	.18	1.24	2.33
	Metkovic-2	26		52	.12	.18	1.24	2.33
	Skadarsko Jezero-1	6		12	.18	.18	1.24	2.33
	Skadarsko Jezero-2	20		40	.12	.12	1.24	3.00
	Skopje-1	25		50	.12	.24	1.29	2.25
	Skopje-2	41		82	.18	.29	1.47	2.60
	Igoumenitsa-1	5		10	.06	.06	1.06	2.00
	Igoumenitsa-2	18		36	.18	.18	1.24	2.33
<i>Rana lessonae</i> *								
	Zagreb-1	0	17	17	.18	.18	1.18	2.00
	Zagreb-2	1	17	19	.18	.18	1.18	2.00
	San Dona-1	1	30	32	.12	.29	1.35	2.20
	San Dona-2	1	42	44	.06	.47	1.53	2.13
Italian nonhybrid *								
	Lavello-1	34	11	79	.18	.35	1.41	2.17
	Lavello-2	3	36	42	.18	.18	1.24	2.33
	Tarsia-1	2	33	37	.29	.29	1.41	2.40
	Tarsia-2	0	29	29	.29	.29	1.41	2.40
	Catania-1	23	58	104	.06	.24	1.24	2.00
	Catania-2	0	31	31	0	0	1.00	-
Yugoslavian taxon								
	Skadarsko Jezero-1	31	62		0	0	1.00	-
	Skadarsko Jezero-2	9	18		0	0	1.00	-
Greek taxon								
	Igoumenitsa-1	27	54		.06	.12	1.12	2.00
	Igoumenitsa-2	11	22		0	.12	1.12	2.00

Table 5 (continued).

Taxon	NH	NG	Polymorphism		Number of alleles	
Locality			P	P'	A	A'
(2) All genomes combined in hybridogenetic hybrids:						
Rana esculenta						
Zagreb-1	17	34	.35	.35	1.53	2.50
Zagreb-2	17	34	.35	.41	1.59	2.43
San Dona-1	30	60	.35	.41	1.71	2.57
San Dona-2	42	84	.35	.53	1.77	2.33
Italian hybrid						
Lavello-1	11	22	.53	.53	1.65	2.22
Lavello-2	36	72	.53	.53	1.65	2.22
Tarsia-1	33	66	.53	.53	1.71	2.33
Tarsia-2	29	58	.53	.59	1.77	2.30
Catania-1	58	116	.41	.41	1.53	2.29
Catania-2	31	62	.41	.41	1.41	2.00
(3) ridibunda genomes in hybridogenetic hybrids:						
Rana esculenta						
Zagreb-1	17	17	.12	.12	1.121	2.00
Zagreb-2	17	17	.12	.12	1.126	2.00
San Dona-1	30	30	0	0	1.005	-
San Dona-2	42	42	0	0	1.005	-
Italian hybrid						
Lavello-1	11	11	.06	.06	1.061	2.00
Lavello-2	36	36	.06	.06	1.061	2.00
Tarsia-1	33	33	0	0	1.000	-
Tarsia-2	29	29	0	0	1.000	-
Catania-1	58	58	0	0	1.000	-
Catania-2	31	31	0	0	1.000	-

Table 6. Polymorphism and number of alleles : Summary.
The estimates are based on electrophoretic data using 17 structural gene loci (MPI, 6PGDH and Alb are omitted; Appendix 2).

P = proportion of polymorphic loci (frequency of most common allele ≤ 0.95)

P' = proportion of heteroallelic loci (independent of allele frequencies)

TA = total number of alleles detected

A = mean number of alleles per locus

A' = mean number of alleles per heteroallelic locus

* = calculated from pooled samples of nonhybrids and nonhybrid genomes in hybrids

m = arithmetic mean of sample values; SD = empirical standard deviation of sample values; wm = weighted mean (by number of genomes sampled).

Localities are listed in Appendix 1 and mapped in Fig. 1.

Taxon Locality	Number of sites	Number of genomes	Polymorphism (in %)						Number of alleles										
			P			P'			TA	A			A'						
			m	range	SD	wm	m	range		SD	wm	m	range	SD	wm				
(1) Genomes of Mediterranean species:																			
Rana ridibunda	10	418	17	6-29	8	17	21	6-35	9	23	30	1.27	1.06-1.47	.10	1.30	2.32	2.00-3.00	.31	2.36
Rana lessonae *	4	112	14	6-18	6	12	28	18-47	14	33	30	1.31	1.18-1.53	.17	1.37	2.08	2.00-2.20	.10	2.11
Italian nonhybrid *	6	322	17	0-29	12	15	23	0-35	12	25	31	1.29	1.00-1.41	.16	1.20	2.26	2.00-2.40	.17	2.19
Yugoslavian taxon	2	80	0	0	0	0	0	0	0	0	17	1.00	1.00	0	1.00	-	-	-	-
Greek taxon	2	76	3	0-6	4	4	12	12	0	12	20	1.12	1.12	0	1.12	2.00	2.00	0	2.00
(2) All genomes combined in hybridogenetic hybrids:																			
Rana esculenta	4	212	35	35	0	35	43	35-53	8	45	35	1.65	1.53-1.77	.11	1.69	2.46	2.33-2.57	.10	2.44
Italian hybrid	6	396	49	41-53	6	48	50	41-59	7	49	37	1.62	1.41-1.77	.13	1.61	2.23	2.00-2.33	.12	2.08
(3) ridibunda genomes in hybridogenetic hybrids:																			
Rana esculenta	4	106	6	0-12	7	4	6	0-12	7	4	19	1.06	1.00-1.12	.07	1.04	2.00	2.00	0	2.00
Italian hybrid	6	198	2	0-6	3	1	2	0-6	3	1	19	1.02	1.00-1.06	.03	1.01	2.00	2.00	0	2.00

their occurrence may reflect some amount of recent migration between the largely parapatric areas of Rana lessonae and the Italian nonhybrid (Fig. 1,3), followed by hybridization and backcrosses between these two related taxa. Comparison of the P' and P values among samples within a taxon (Table 5) shows the same pattern in Rana ridibunda and in the Italian nonhybrid: decreasing from highest at Zagreb through Skopje, Metković and Skadarsko Jezero to lowest at Igoumenitsa for the former, higher values for the peninsular than for the Sicilian samples of the latter.

The average number of alleles per locus (A over all loci, A' per heteroallelic locus; Tables 5 and 6) and the total number of alleles detected in a taxon (Table 6) reveal similar patterns in genic variability among the Mendelian taxa compared: The Yugoslavian and the Greek taxon harbor the lowest amount of diversity; Rana ridibunda, the Italian nonhybrid and Rana lessonae the highest. Again, the many rare alleles found in Italian nonhybrid genomes and especially in Rana lessonae genomes at San Donà account for the high mean A values and total number of alleles found for these two taxa (Table 6).

For the hybridogenetic hybrids discussed, all alleles that have been found combined in individuals were used to calculate, for comparison, relative values of polymorphism and allele numbers present in hybrids (Tables 5,6). These values averaged generally higher than those for any Mendelian taxon, especially P , P' , A and total number of alleles (Table 6). Specifically, all these measures of variability for groups of hybrid individuals (including the A' values, which are encompassed by A' values in Rana ridibunda populations) were higher, at each site, than those calculated for the syntopic non-ridibunda parent (Table 5). This is not surprising, since it simply reflects the alleles in ridibunda genomes of hybrids that are not shared by the syntopic non-ridibunda parent; this diversity is added to all variation already available from the local gene pool of the host species. The relative P and P' values averaged higher in the Italian hybrid than in Rana esculenta (Table 6); P , P' and A were larger for hybrids allopatric to Rana ridibunda than for hybrids from Zagreb (Table 5); the mean number of alleles per heteroallelic locus was, however, higher in Rana esculenta than in the Italian hybrid (A' , Table 5).

Polymorphism and number of alleles were also calculated for the clonally inherited ridibunda genomes of hybridogenetic hybrid lineages (Tables 5,6).

All values obtained are low, generally lower than those for the Mendelian taxa except the least variable Yugoslavian and Greek taxa. Specifically, the local (Table 5) and overall (Table 6) variability averaged higher in ridibunda genomes present in Mendelian Rana ridibunda populations than in ridibunda genomes present in hemiclinal hybrid lineages; even at the Zagreb sites, where hybrids are syntopic with Mendelian Rana ridibunda populations, this difference is shown in P, P' and A (Table 5).

Heterozygosity

The observed heterozygosity reflects how the locally present genic variation is organized into individual genotypes. For the water frogs discussed, the observed individual heterozygosities per locus and sample are reported in Table 7; mean observed individual heterozygosities over all loci are given for samples in Table 8, and summarized over all samples of the taxa compared in Table 9.

Among populations of the Mendelian taxa, observed mean heterozygosity was highest in Rana ridibunda, followed in decreasing sequence by Italian nonhybrid, Greek taxon and Yugoslavian taxon (Tables 8,9). This parallels the pattern found in polymorphism and number of alleles. Among samples of a taxon (Table 8), mean heterozygosity decreased in Rana ridibunda, in all combinations of loci for which it was calculated, in the region sequence of Zagreb, Skopje, Igoumenitsa, Skadarsko Jezero and Metković; in the Italian nonhybrid, the mainland Lavello sample gave higher values than the island sample from Catania. This parallels data obtained for polymorphism and number of alleles.

While there was some variation in the observed heterozygosity between taxa and regions in Mendelian populations, hybridogenetic hybrids revealed very different values (Tables 7,8,9): their individual heterozygosity without exception was extremely high, much higher than any value found in any Mendelian population. This pattern is obvious in the summary in Table 9: The overall mean of individual heterozygosity observed in the hybridogenetic hybrid Rana esculenta (34 %) is larger than that observed in the Mendelian species Rana ridibunda by a factor of 5.5, in the Italian hybrid (42 %), by a factor of 6.6; the differences from the other Mendelian taxa, with lower mean heterozygosities than Rana ridibunda, are even more extreme. Within the

Table 7. Heterozygosity: values for 20 structural gene loci among samples.
 Observed frequency of individuals heterozygous per locus. N = number of individuals, G = number of genomes sampled;
 - = not determined (Appendix 2). Localities are listed in Appendix 1 and mapped in Fig. 1.

Taxon Locality	N	G	AAT-1	AK	CK	GDH	GPI	IDH-1	IDH-2	LDH-1	LDH-2	MDH-1	MDH-2	MPI	Pep-2	PGM-2	6PGDH
(1) Mendelian populations:																	
Rana ridibunda	21	42	0	0	0	0	.33	0	0	.43	.10	0	0	.57	.10	.43	.43
Zagreb-1	21	42	.05	0	0	0	.57	0	0	.57	.14	0	0	.43	.19	.14	.43
Zagreb-2	26	52	.12	0	0	0	.50	0	0	0	0	0	0	0	0	.08	0
Metkovic-1	26	52	.12	0	0	0	.58	0	0	0	0	0	0	0	0	.04	0
Metkovic-2	6	12	0	0	0	0	.33	0	0	0	0	0	0	0	0	.50	0
Skadarsko Jezero-1	20	40	0	0	0	0	.60	0	0	0	0	0	0	.10	0	.35	0
Skadarsko Jezero-2	25	50	.04	0	0	0	.60	0	0	0	0	0	0	.32	0	.28	.16
Skopje-1	41	82	.12	0	0	0	.59	0	0	.02	0	0	0	.32	.10	.29	.15
Skopje-2	5	10	0	0	0	0	.40	0	0	0	0	0	0	.60	0	0	0
Igoumenitsa-1	18	36	.28	0	0	0	.44	0	0	0	0	0	0	.50	0	.44	.06
Igoumenitsa-2																	
Italian nonhybrid																	
Lavello-1	34	68	.32	0	.35	0	0	.15	0	0	0	0	0	-	0	0	-
Catania-1	23	46	0	0	0	.02	0	0	0	0	0	0	0	-	.11	0	.02
Yugoslavian taxon																	
Skadarsko Jezero-1	31	62	0	0	0	0	0	0	0	0	0	0	0	.07	0	0	-
Skadarsko Jezero-2	9	18	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	-
Greek taxon																	
Igoumenitsa-1	27	54	.04	0	0	.11	0	0	0	0	0	0	0	.22	0	0	-
Igoumenitsa-2	11	22	0	0	0	.09	0	0	0	0	0	0	0	.18	.09	0	-
(2) Hybridogenetic hybrids as individuals:																	
Rana esculenta																	
Zagreb-1	17	34	1	0	0	1	.47	0	0	1	0	0	0	1	.94	1	1
Zagreb-2	17	34	1	0	0	1	.47	.06	0	1	0	0	0	1	1	.94	1
San Donà-1	30	60	.97	0	0	1	1	0	0	1	0	.03	0	1	1	1	1
San Donà-2	42	84	.95	0	.02	1	.98	.02	.02	1	0	0	0	1	.98	1	1
Italian hybrid																	
Lavello-1	11	22	.46	0	.91	1	1	.09	0	1	0	0	0	1	1	1	1
Lavello-2	36	72	.25	0	.81	1	1	.11	0	1	0	0	0	1	1	1	1
Tarsia-1	33	66	.33	0	.70	1	.94	0	0	1	0	.24	0	1	1	1	1
Tarsia-2	29	58	.28	0	.90	1	.86	.03	0	1	0	.28	0	1	1	1	1
Catania-1	58	116	0	0	0	1	.93	0	0	1	0	1	0	1	1	1	1
Catania-2	31	62	0	0	0	1	1	0	0	1	0	1	0	1	1	1	1

Table 7 (continued).

Taxon Locality	N	G	SOD	MProt1	MProt2	MProt3	Alb
(1) Mendelian populations:							
Rana ridibunda							
Zagreb-1	21	42	0	0	0	0	-
Zagreb-2	21	42	0	0	0	0	-
Metković-1	26	52	1	0	0	0	-
Metković-2	26	52	1	0	0	0	-
Skadarsko Jezero-1	6	12	17	0	0	0	-
Skadarsko Jezero-2	20	40	0	0	0	0	-
Skopje-1	25	50	0	0	0	0	-
Skopje-2	41	82	0	0	0	0	-
Igoumenitsa-1	5	10	0	0	0	0	-
Igoumenitsa-2	18	36	0	0	0	0	-
Italian nonhybrid							
Lavello-1	34	68	0	.03	0	0	.15
Catania-1	23	46	0	0	0	0	.52
Yugoslavian taxon							
Skadarsko Jezero-1	31	62	0	0	0	0	-
Skadarsko Jezero-2	9	18	0	0	0	0	-
Greek taxon							
Igoumenitsa-1	27	54	0	0	0	0	-
Igoumenitsa-2	11	22	0	0	0	0	-
(2) Hybridogenetic hybrids as individuals:							
Rana esculenta							
Zagreb-1	17	34	0	0	0	0	1
Zagreb-2	17	34	0	0	0	0	1
San Dona-1	30	60	0	0	0	0	1
San Dona-2	42	84	0	0	0	0	1
Italian hybrid							
Lavello-1	11	22	0	1	0	0	1
Lavello-2	36	72	0	1	0	0	1
Tarsia-1	33	66	0	1	0	0	1
Tarsia-2	29	58	0	1	0	0	1
Catania-1	58	116	0	1	0	0	1
Catania-2	31	62	0	1	0	0	1

Table 8. Heterozygosity: Sample means.

Mean proportion of observed individual heterozygosities per sample (H), estimated by protein electrophoresis. Only samples of ≥ 5 individuals are included. N = number of individuals; (a) = MPI, 6PGDH and Alb omitted; (b) = MPI and 6PGDH omitted; (c) = 6PGDH and Alb omitted; (d) = Alb omitted; - = not calculated (missing data). Localities are listed in Appendix 1 and mapped in Fig. 1.

Taxon	N	H (in %)			
Locality		(a)	(b)	(c)	(d)
		17 loci	18 loci	18 loci	19 loci
(1) Mendelian populations:					
Rana ridibunda					
Zagreb-1	21	8.2	-	10.9	12.6
Zagreb-2	21	9.8	-	11.6	13.3
Metković-1	26	4.1	-	3.9	3.7
Metković-2	26	4.4	-	4.1	3.9
Skadarsko Jezero-1	6	5.9	-	5.6	5.3
Skadarsko Jezero-2	20	5.6	-	5.8	5.5
Skopje-1	25	5.7	-	7.1	7.6
Skopje-2	41	6.6	-	8.0	8.4
Igoumenitsa-1	5	2.4	-	5.6	5.3
Igoumenitsa-2	18	6.8	-	9.2	9.1
Italian nonhybrid					
Lavello-1	34	5.0	5.6	-	-
Catania-1	23	0.8	3.6	-	-
Yugoslavian taxon					
Skadarsko Jezero-1	31	0	-	0.4	-
Skadarsko Jezero-2	9	0	-	0.6	-
Greek taxon					
Igoumenitsa-1	27	0.9	-	2.1	-
Igoumenitsa-2	11	1.1	-	2.0	-
(2) Hybridogenetic hybrids:					
Rana esculenta					
Zagreb-1	17	31.8	35.6	35.6	39.0
Zagreb-2	17	32.2	35.9	35.9	39.3
San Donà-1	30	35.3	38.9	38.9	42.1
San Donà-2	42	35.1	38.7	38.7	41.9
Italian hybrid					
Lavello-1	11	43.9	47.0	47.0	49.8
Lavello-2	36	42.2	45.4	45.4	48.3
Tarsia-1	33	42.4	45.6	45.6	48.5
Tarsia-2	29	43.2	46.4	46.4	49.2
Catania-1	58	40.8	44.1	44.1	47.0
Catania-2	31	41.2	44.4	44.4	47.4

Table 9. Heterozygosity: Summary.

Mean observed individual heterozygosity of Mendelian taxa and of hybridogenetic hybrids of water frogs, based on electrophoretic data for 17 structural gene loci (MPI, 6PGDH, Alb are omitted; Appendix 2). Only samples of ≥ 5 individuals are included. Localities are listed in Appendix 1 and mapped in Fig. 1. SD = empirical standard deviation.

Taxon	Number of samples	Number of indi- viduals	Mean proportion of loci heterozygous per individual (in %)				Grand mean of all individuals
			Sample values				
			mean	range	SD		
(1) Mendelian taxa:							
Rana ridibunda	10	209	5.6	2.4 - 9.8	2.1	6.2	
Italian nonhybrid	2	57	2.9	0.8 - 5.0	3.0	3.6	
Yugoslavian taxon	2	40	0	0	0	0	
Greek taxon	2	38	1.0	0.9 - 1.1	0.1	0.9	
(2) Hybridogenetic hybrids:							
Rana esculenta	4	106	33.6	31.8 - 35.3	1.9	34.2	
Italian hybrid	6	198	42.3	40.8 - 43.9	1.2	41.9	

hybridogenetic lineages, the samples allopatric to Rana ridibunda gave higher mean heterozygosity values than the Zagreb samples sympatric with it, and the Italian hybrid gave higher values than Rana esculenta (Table 8).

Clonal diversity in ridibunda genomes of hybridogenetic hybrid lineages

Electrophoretic data for all 20 structural gene loci discussed were used to estimate clonal diversity in the ridibunda genomes of the hybridogenetic hybrid lineages. A total of 17 different ridibunda genome clones was detected among the 304 hybrids studied. These 17 haplotypes (A-Q) are electrophoretically defined in Table 10. The alleles IDH-1 a (Zagreb-2, Tarsia-2) and IDH-2 a (San Donà-2) of hybrids, not assignable to a parental genome, were not included, and at Zagreb the GPI f allele was assumed to be a lessonae allele in each hybrid (Appendix 4); so the 17 ridibunda haplotypes distinguished in hybrids represent a minimum of hemiclones for the electrophoretic data obtained.

The clonal diversity locally observed among ridibunda genomes of coexisting hybridogenetic hybrid lineages is presented in Table 11. The following patterns are seen.

(1) A discrete decrease in clonal diversity among ridibunda genomes of hybrids at a site occurred between the area of sympatry with Rana ridibunda (Zagreb) and the regions where hybrids live outside of the present Rana ridibunda range (all Italian localities). Among the 34 hybrids from the two Zagreb sites, 10 different ridibunda hemiclones were detected; at each site, 8 hemiclones were found, about half the number of hybrid individuals collected. Among the 270 hybrids from the 8 sites of 4 Italian regions, 7 different hemiclones were found, none of which is shared with Zagreb; per site, 1 to 4 distinguishable hemiclones were present in the Italian samples.

(2) No continuous decrease of clonal diversity among locally present ridibunda genomes of hybrids along a north-south gradient of increasing distance from the Rana ridibunda range was found. At both San Donà sites, separated by about 100 km (largely a lowland plain area, with many water bodies) from the present Rana ridibunda range, only 2 hemiclones were found (K and L), while at the Lavello sites, as far south as the Basilicata region (Fig. 1) and about 650 km south of San Donà by land, 4 different

Table 10. Clones of ridibunda genomes in hybridogenetic hybrid lineages. Haplotypes of the clonally inherited ridibunda genomes of hybrids detected by electrophoresis among 20 structural gene loci. For the 14 loci not included, all 17 clones share the same electrophoretic alleles (AK a, CK b, α -GDH a, IDH-1 b, IDH-2 b, LDH-1 c, LDH-2 a, MDH-1 b, MDH-2 a, Pep-2 b, SOD a, MProt1 b, MProt2 a, MProt3 a). See text for assumptions used.

Clone	Loci varying in ridibunda genomes of hybrids						Localities
	AAT-1	GPI	MPI	PGM-2	6PGDH	Alb	
A	e	d	c	d	d	c	Zagreb-1+2
B	e	d	c	d	d	b	Zagreb-1+2
C	e	a	c	b	d	b	Zagreb-1+2
D	e	a	c	d	e	c	Zagreb-1+2
E	e	d	c	b	d	b	Zagreb-1+2
F	e	a	c	d	d	b	Zagreb-1+2
G	e	d	a	b	d	c	Zagreb-1
H	e	d	a	d	d	b	Zagreb-1
I	e	d	c	d	e	b	Zagreb-2
J	e	d	c	d	d	h	Zagreb-2
K	e	a	c	b	e	h	San Donà-1+2
L	e	a	c	b	d	h	San Donà-1+2
M	e	a	c	b	d	i	Lavello-1+2, Tarsia-1+2
N	j	a	c	b	e	i	Lavello-1+2
O	e	a	c	b	e	i	Lavello-1+2
P	j	a	c	b	d	i	Lavello-1
Q	e	a	c	d	d	i	Catania-1+2

Table 11. Clonal diversity at a site in hybridogenetic hybrid lineages. Minima of locally present haplotypes of the clonally inherited ridibunda genomes in hybrid lineages, detected by electrophoresis among 20 structural gene loci. Approximate shortest terrestrial distances from the present autochthonous Rana ridibunda range are indicated. Genetic backgrounds of the ridibunda genomes in hybrids are Rana lessonae genomes at Zagreb and San Donà, Italian nonhybrid genomes at Lavello, Tarsia and Catania. The haplotypes listed are electrophoretically defined in Table 10. N = total number of hybrids collected at a site. * = 1 individual possibly triploid (Appendix 3). ** = separated from peninsular Italy by the Strait of Messina.

Region	Distance from <u>Rana ridibunda</u> range (km)	Total number of clones observed in region	Site	N	Number of clones observed at site	Haplo-types	Number of individuals	Frequency
Zagreb	sympatric	10	1	17	8	A	5	.29
						B	1	.06
						C	4	.24
						D	2	.12
						E	2	.12
						F	1	.06
						G	1	.06
						H	1	.06
			2	17	8	A	2	.12
						B	4	.24
						C	1	.06
						D	3	.18
						E	1	.06
						F	1	.06
						I	3 *	.18
						J	2	.12
San Donà	100	2	1	30	2	K	20	.67
						L	10	.33
			2	42	2	K	41	.98
						L	1	.02
Lavello	750	4	1	11	4	M	3	.27
						N	3	.27
						O	3	.27
						P	2	.18
			2	36	3	M	28	.78
						N	7	.19
Tarsia	950	1	1	33	1	M	33	1.00
			2	29	1	M	29 *	1.00
Catania	1250 **	1	1	58	1	Q	58	1.00
			2	31	1	Q	31	1.00

hemiclones were distinguished (M,N,O,P). Moreover, neither of the 2 hemiclones present at San Donà and none of the 4 present at Lavello is shared with Zagreb, and no hemiclones are present at both San Donà and Lavello. On the other hand, judged by electrophoretic data available, the most frequent hemiclone from Lavello (M) is the same as the single hemiclone found at the Tarsia sites of the Calabria region (Fig. 1). Finally, a single, unique hemiclone was observed for Sicily (Q at Catania).

(3) The amount of clonal diversity at a site among ridibunda genomes of hybridogenetic hybrid lineages was lower at Zagreb than that among ridibunda genomes of syntopic Mendelian Rana ridibunda populations (Table 5); in hybridogenetic hybrid lineages of the Italian region, which are allopatric to Rana ridibunda, clonal diversity was distinctly lower than that of Mendelian Rana ridibunda populations.

In sum, the following patterns of genic diversity are observed in the water frog samples discussed. Among populations of Mendelian taxa, variability as judged by proportion of polymorphic loci, number of alleles and individual heterozygosity was usually highest in Rana ridibunda, intermediate in the Italian nonhybrid and in Rana lessonae, and lowest in the Greek and the Yugoslavian taxon. Within the Mendelian taxa, Rana ridibunda was more variable at the inland localities of Zagreb and Skopje than in the coastal areas of Metković, Skadarsko Jezero and Igoumenitsa; and the Italian nonhybrid was most variable at Tarsia, slightly less at Lavello, but in both of these mainland regions more variable than on the island of Sicily.

In hybridogenetic hybrid lineages, clonal diversity among the locally present ridibunda genomes was restricted compared to that of Mendelian Rana ridibunda populations, especially outside of the present Rana ridibunda range, but even in syntopy with that species. On the other hand, the hybridogenetic hybrids revealed a very high degree of individual heterozygosity (between 30 and 50 %) throughout all regions, differing by a factor of up to 6.6 (Italian hybrid) from the values found in Rana ridibunda, and by even more from the less variable other Mendelian taxa.

Clonal diversity at a site was highest in hybrid lineages sympatric with Rana ridibunda; in allopatry with this species it did not, however, continuously decrease with increasing distance from the Rana ridibunda area, but rather had a maximum in the southern Italian Lavello region; no hemiclones

were shared between the hybrid lineages sympatric with and those allopatric to Rana ridibunda, none between hybrids from the Po valley and from peninsular Italy, and none between those from Sicily and from mainland Italy.

3.2 SOURCES OF HETEROZYGOSITY IN HYBRIDS

The genetic diversity in hemiclinal hybrid lineages at a site is present in two different ways: as genic heterozygosity in individual hybrids, and as interclonal differences among coexisting hemiclones.

The hybridogenetic hybrids are extremely heterozygous in somatic tissues (see 3.1). This is clearly a result of their interspecific hybrid status. Each individual possesses a genome that originally stemmed from the species Rana ridibunda plus a genome of the non-ridibunda parental species syntopic with the hybrids (Rana lessonae or Italian nonhybrid). The ridibunda genome is clonally inherited from hybrid ancestors through hybridogenetic gametogenesis, the non-ridibunda genome is drawn in each generation from the gene pool of the host species by hybrid $\times$ nonhybrid mating (Appendix 5). Hybridogenesis so maintains a permanent " F_1 state", without segregational load, in hybrid lineages. The high heterozygosity of a hybrid reflects the amount of genetic difference between its two specifically distinct parental genomes.

To illustrate this pattern in a summary of the electrophoretically detected genetic differences, the allelic frequencies in Tables 3 and 4 were used to calculate genetic distances (Nei 1972, 1973, standard distance, and Rogers 1972); the obtained distances between local samples of each of 7 OTUs (Rana ridibunda, ridibunda genomes in hybrids sympatric with Rana ridibunda, ridibunda genomes in hybrids allopatric to Rana ridibunda, Rana lessonae, Italian nonhybrid, Yugoslavian taxon, Greek taxon) were averaged (Table 12). The highest average value found, both in Nei and Rogers distances, is that between the Italian nonhybrid and ridibunda genomes of hybrids allopatric to Rana ridibunda; all other comparisons between ridibunda genomes of hybrids and genomes of the non-ridibunda parental species also gave relatively large distances. At a site, genetic distances between ridibunda

genomes in hybrids and genomes of the syntopic host parental species for Rana esculenta ranged from 0.33 to 0.41 (Nei) and from 0.39 to 0.45 (Rogers); for the Italian hybrid, from 0.51 to 0.54 (Nei) and from 0.53 to 0.56 (Rogers). Among the 20 loci studied, the 3 not included for genetic distance calculation because of missing data (MPI, 6PGDH, Alb) would have further increased these distances, since at each of them, for all localities, no alleles are shared between ridibunda genomes of hybrids and genomes of the syntopic non-ridibunda parental species.

The proportion of loci that proved to be diagnostic between the two parental genomes of hybrids is high: 40 % for Rana esculenta (for α -GDH, LDH-1, MPI, 6PGDH and Alb no alleles were shared between genomes of the parental species; for AAT-1, Pep-2, PGM-2 and at San Donà for GPI also the parental genomes differed in most cases); and 45 % for the Italian hybrid (no alleles shared for α -GDH, LDH-1, MPI, Pep-2, PGM-2, 6PGDH, Alb and at Catania also MDH-1, in most cases different alleles for GPI and MProt1). This accounts for the fixed heterozygosities of hybrids at these loci: per locus heterozygosity commonly was 1.0 in hybrid samples (Table 7); high heterozygosity in a hybrid sample sometimes parallels absence of any interindividual variation, as exemplified by the Catania-2 sample (Table 7).

That the Italian hybrid, completely allopatric to Rana ridibunda (Fig. 1), showed an even higher degree of heterozygosity than Rana esculenta (Table 9) is due mainly to the larger genetic difference between Rana ridibunda and the Italian nonhybrid than between Rana ridibunda and Rana lessonae (illustrated by the genetic distances in Table 12). Obviously, the interspecific genetic differences between parental genomes of hybrids also account for the higher polymorphism and allele number values obtained for hybrids as groups of individuals (Tables 5,6): To the variability locally available from the syntopic parent's gene pool are added all alleles that are not shared between this gene pool and the ridibunda genomes of hybrids. Again, the generally higher relative P, P' and A values for the Italian hybrid compared to Rana esculenta (Tables 5,6) reflect the usually higher genic variability of the Italian nonhybrid relative to Rana lessonae in the samples studied.

Most of the fixed heterozygosity level of hybrids obviously is pre-established by interspecific hybridization of Rana ridibunda, when new hemiclinal hybrid lineages are brought into existence; from then on it reflects the genetic differences (Table 12) between Rana ridibunda and the other

species that supplies a genome in a given generation of hybridogenetic lineages. After hemiclone formation, this fundamental level of heterozygosity may be changed not only by matings of hybrids with different individuals, but also by genetic changes occurring in the clonally inherited ridibunda genome (see 3.4): increased by novel mutations providing functional gene products; decreased by mutations leading to nonfunctional gene products or (if this occurs) by recombination between the parental genomes in hybrids.

3.3 SOURCES OF CLONAL DIVERSITY AMONG HYBRID LINEAGES SYMPATRIC WITH RANA RIDIBUNDA

The largest amount of clonal diversity present among hybrid lineages at a site was found in the area of sympatry with Rana ridibunda (see 3.2). All data are consistent with the interpretation that the unique source of clonal diversity in this area is multiple hybridizations, Rana ridibunda × Rana lessonae, that resulted in hemiclone syntheses.

All alleles found in hybrids at the Zagreb sites (an area of sympatry) that were assignable to ridibunda genomes were also observed, with one possible exception, in the local Rana ridibunda populations, including those multiple alleles of polymorphic loci that distinguish the 10 ridibunda genome clones found among hybrids from Zagreb (Table 10): GPI a, d, MPI a, c, PGM-2 b, d, 6PGDH d, e, Alb b, c. The h Alb allele, found in the ridibunda genomes of two hybrids from Zagreb-2 (haplotype J, Table 10), was not clearly distinguishable by electrophoresis from the b allele in Rana ridibunda individuals, but probably also occurs in syntopic Rana ridibunda (Appendix 2).

For the Zagreb samples, allele frequencies were determined for 8 locally varying loci in Rana ridibunda (Table 3). For 6 of them (AAT-1, GPI, LDH-2, Pep-2, PGM-2, 6PGDH) the observed frequencies of ridibunda alleles among hybrid hemiclones do not differ significantly from frequencies in the syntopic Rana ridibunda populations ($p > .10$ for each comparison, contingency table analyses). For LDH-1 and MPI, however, the observed frequencies of

ridibunda alleles among hybrids deviated considerably from those in the syntopic Rana ridibunda population in both sample replicates. The LDH-1 a allele, frequent in Rana ridibunda (Zagreb-1: .60, Zagreb-2: .52), was not observed in any hybrid. Similarly, the MPI a allele, frequent in Rana ridibunda (Zagreb-1: .57, Zagreb-2: .36), was much rarer than the c allele in ridibunda genomes of hybrids (Zagreb-1: .12, Zagreb-2: 0). Each of these differences is significant (2×2 table analysis: X^2 for LDH-1 = 17.6 at Zagreb-1, 14.2 at Zagreb-2; for MPI = 10.1 at Zagreb-1, 8.1 at Zagreb-2; $p < .01$ for each comparison). This difference may reflect immigration of hybrids from different breeding populations, rather than origination of all of them from the local Rana ridibunda population sampled (Appendix 5). On the other hand, since at both loci allele frequencies are similar for Rana ridibunda and the hybrids deviate in the same direction in both sample replicates, it seems plausible that ridibunda alleles interact differently with other ridibunda alleles or genomes and with lessonae alleles or genomes. Consistent with this hypothesis is the observation that for both loci the allele overrepresented in hybrids from Zagreb (LDH-1 c, MPI c) is the only one found in ridibunda genomes of hybrids from Italy, although at both loci Rana ridibunda often is polymorphic or even locally fixed for alleles not found in hybrids from Italy (LDH-1 a, MPI a: Table 3 and Appendix 2). Interestingly, for both loci the a allele absent in hybrid lineages from Italy is the ridibunda allele found in the hybridogenetic Rana ridibunda $\times$ Rana perezi hybrid lineages (Appendix 2).

3.4 A COMPLEX PATTERN OF CLONAL DIVERSITY AMONG HYBRID LINEAGES IN ITALY

While all clonal diversity detected among coexisting hybrid lineages in the area of sympatry with Rana ridibunda consistently can be attributed to multiple hybrid syntheses, the interpretation of the observed clonal diversity in hybrid lineages from the Italian regions, which are outside of the Rana ridibunda range, remains more ambiguous.

Sources of local clonal diversity

In two of the four Italian regions studied multiple coexisting hybrid hemiclones were detected (Table 11). At San Donà, two haplotypes of the clonally inherited ridibunda genomes (K,L) were found in both sample replicates, although in different frequencies; hemiclone L was less common at both sites, but especially rare at San Donà-2. The two hemiclones differ at the 6PGDH locus (alleles d and e: Table 10); their coexistence is directly confirmed by testis extracts of two hybrids from San Donà-1 (Table 20 in Appendix 4), in which only the d and e allele, respectively, was expressed. Both alleles occur also in Rana ridibunda populations (Table 3 and Appendix 2); the e allele characterizing the more common hemiclone K was more frequent in the northernmost (Zagreb) populations of Rana ridibunda, nearest to San Donà and within the Rana lessonae range, than in the other regions. This suggests that multiple immigration of hybrid lineages that originated in the present sympatry area of Rana ridibunda and Rana lessonae plausibly accounts for the coexistence of the two hemiclones at San Donà.

At Lavello, four ridibunda genome haplotypes were distinguishable in hybrids (M,N,O,P: Table 11), represented at about equal frequencies in the small sample from Lavello-1; at Lavello-2, M vastly predominates and P was not found. The alleles discriminating these hemiclones are AAT-1 e and j, and 6PGDH d and e (Table 10), found in all four possible combinations. While the 6PGDH d and e alleles occur in Rana ridibunda (these two alleles distinguish the hybrid hemiclones of San Donà) and AAT-1 e is the common allele of Rana ridibunda, the j AAT-1 allele has not so far been found in Rana ridibunda from any part of its range (Appendix 2). This finding cannot easily be explained. Multiple immigration of hybrid lineages could readily account for the coexistence of two hemiclones distinguished by the 6PGDH d and e alleles, and it can be speculated that the AAT-1 j allele represents a successful novel mutation that occurred in a clonally inherited ridibunda genome of a hybrid ancestor. This would, however, lead only to 3 hemiclones (M, O and either N or P; Table 10); it seems improbable that the same novel mutation independently occurred (and was established) twice in hybrid lineages (changing hemiclone M into P, and separately, O into N). Three alternative explanations suggest themselves: (1) Experimental error: This is rejected because the products of the AAT-1 e and j alleles have such different mobilities that a mis-scoring can be excluded (Appendix 2); the assignment of both alleles to ridibunda genomes of coexisting hybrids is cer-

tain (Appendix 4). The presence of the two different 6PGDH alleles in ridibunda genomes of hybrids is directly demonstrated by testis extracts of two males (d in 919, e in 868: Table 20 in Appendix 4); at the AAT-1 locus, the ridibunda genome of the same male 919 contained the e allele (hemiclone M), that of male 868 the j allele (hemiclone N). The occurrence of hemiclone O is also certain (a mis-scoring can be excluded for both alleles characterizing it). On the other hand, it is not certain that the rarest hemiclone P may not represent an artefact due to the invisibility of the faint band revealing the 6PGDH e allele (Appendix 2) and thus may be identical to hemiclone N, although this seems unlikely, since the electrophoretic pattern was repeatedly observed in the two hybrids of hemiclone P. (2) Multiple immigrations of hybrid lineages, originated in parts of the Rana ridibunda area where the AAT-1 j allele does occur at present but has not been found so far. (3) Multiple immigrations of hybrid lineages in the past, reflecting a different former allele distribution in Rana ridibunda populations then existing in the area of hybrid origin. It cannot presently be decided whether any and which of these explanations account for the clonal diversity observed at Lavello.

Did clonally inherited genomes evolve after clone formation?

No conclusive evidence for mutations in the clonally inherited ridibunda genomes of hybrid lineages is provided by the electrophoretic data reported here. No silent allele (reflected in exclusive expression of a non-ridibunda parental allele at a locus usually heterozygous in hybrids) was detected in any of the 270 hybridogenetic hybrids examined from Italy.

In looking for novel mutations providing functional translation products, three alleles have to be considered. The AAT-1 j allele of ridibunda genomes in hybrids from Lavello is not known in Rana ridibunda; as discussed above, however, its occurrence in two different hemiclones (N,P) suggests that multiple immigrations of hybrids (originated from present or past Rana ridibunda populations that did contain the j allele) is more likely to account for its existence than mutation subsequent to hemiclone formation. The Alb j allele common to Italian hybrids from all regions either is a rare allele present in Rana ridibunda (Metković) or is a unique allele; if so, again, its occurrence in 5 different hemiclones (M,N,O,P,Q) is inconsistent with its representing a mutation in clonally inherited genomes (it appears inconceivable that all ridi-

bunda genome alleles differing in the 5 hemiclones also reflect mutations that occurred in hybrid lineages after establishment of the Alb i mutant). Finally, it cannot be excluded that the PGM-2 d allele, distinguishing the Sicilian hemiclone Q from the most widespread and abundant peninsular hemiclone M (Table 10), represents a mutation that led to a translation product mobility convergent to that of an allele common in Rana ridibunda.

Interregional clonal variation: Implications for origin and age of hybridogenetic lineages

A total of 7 ridibunda genome clones was detected in hybrid lineages from Italy (Table 11). Most alleles characterizing these hemiclones (Table 10) also occur in present-day Rana ridibunda populations (Table 3 and Appendix 2). At first glance, this suggests that multiple immigration of hybrid lineages into Italy that originated in the present sympatry area of Rana ridibunda and Rana lessonae is a plausible source of interregional and local clonal diversity in hybrid lineages. This hypothesis is consistent with biogeographical data, which are most simply explained by a recent immigration of hybridogenetic hybrid lineages, synthesized within the present Rana ridibunda range, from the northeast into Italy (Uzzell and Hotz 1979). On the other hand, the following interregional differences between ridibunda genome clones in Italy are striking and suggest a more complex pattern.

(1) In all peninsular and Sicilian samples of the Italian hybrid, its ridibunda genome is characterized by the allele Alb i (haplotypes M,N,O,P,Q), which may either be an allele that presently occurs, although rarely, in Rana ridibunda (Metković), or may be a unique allele (Appendix 2).

(2) In the San Donà samples from the northern edge of the Adriatic Sea, ridibunda genomes of Rana esculenta (haplotypes K,L) contained a different Alb allele (h), never found in the Italian hybrid; it was detected also at Zagreb in Rana esculenta (haplotype J) and probably, but in low frequency, in Rana ridibunda (Appendix 2).

(3) As discussed above, two hybrid hemiclones (N,P), so far found only at Lavello in the Basilicata region, contained the AAT-1 j allele, not known for any Rana ridibunda populations.

(4) The ridibunda genomes of the Italian hybrid from Sicily (haplotype Q) contained the PGM-2 d allele, which is widespread and common in Rana ridibunda and occurs in Rana esculenta from Zagreb (haplotypes A,B,D,F,H,I,J), but was not found in any of the peninsular samples of the

Italian hybrid or of Rana esculenta (San Donà), which instead had the PGM-2 b allele in their ridibunda genomes.

This pattern of interregional variation, as well as the observed pattern of clonal diversity at a site in hybridogenetic lineages (Table 11), is clearly inconsistent with the hypothesis of an exclusive recent immigration of hybrid lineages from the present sympatry area of Rana ridibunda and Rana lessonae into Italy.

Given recent immigration of all ridibunda genomes into Italy, it would be expected that (1) the alleles contained in the clonally inherited genomes of hybrids in Italy are a representative sample of alleles occurring in present-day Mendelian Rana ridibunda populations sympatric with Rana lessonae in a region adjacent to northeastern Italy (Zagreb); (2) that the clonal diversity in hybrid lineages at a site in Italy decreases in a north-south gradient of increasing distance from the Rana ridibunda range (San Donà → Lavello → Tarsia → Catania); and (3) that the hybrid hemiclones present in more southern Italian regions represent a subsample of those present in more northern regions.

Neither of these expectations was fulfilled: (1) There was considerable genetic difference between ridibunda genomes in Rana ridibunda and those in hybrid hemiclones from all Italian regions (Alb, LDH-1, GPI, MPI, PGM-2, partly AAT-1: Tables 3,10, Appendix 2). This is illustrated by genetic distances, calculated from allele frequencies of 19 loci among local samples of Rana ridibunda and ridibunda genomes in hybrid lineages (Table 13), and visualized in a phenogram based on these distances (Fig. 2). All intra-regional genetic distances are small for each OTU; interregional distances within OTUs are also small, generally being larger for hybrid hemiclones than for Rana ridibunda. Genetic distances obtained between Rana ridibunda and hybridogenetic hemiclones can be numerically inflated because of the sudden "freezing" of a single ridibunda haplotype in each event of hybrid synthesis. Nevertheless, used as relative values, they reflect somewhat larger genetic similarity of hybrid hemiclones in Italy to the geographically closer Zagreb samples of Rana ridibunda than to the more southern samples. This is because of geographic variation in allele frequencies of Rana ridibunda at the loci LDH-1, MPI and partly 6PGDH, the alleles relatively more common in the northern, Zagreb, region being those present in ridibunda genomes of hybrids in Italy. On the other hand, the highest genetic dis-

Table 13. Genetic distances among *ridibunda* genomes in *Rana ridibunda* and in hybrids.
Matrix of Nei standard (upper right) and Rogers (lower left) genetic distances between *ridibunda* genomes in samples of Mendelian *Rana ridibunda* populations and of hybridogenetic hybrids, using electrophoretically detected allele frequencies for 19 structural gene loci (Alb omitted; Appendix 2).

OTU	Distances to other OTUs																			
	<i>Rana ridibunda</i>										<i>ridibunda</i> genomes in hybridogenetic hybrids									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<i>Rana ridibunda</i> :																				
1 Zagreb-1	—	.01	.03	.03	.04	.04	.02	.02	.03	.03	.04	.04	.11	.13	.12	.11	.11	.11	.07	.07
2 Zagreb-2	.05	—	.05	.05	.07	.06	.04	.03	.04	.05	.03	.03	.10	.11	.11	.10	.11	.11	.05	.05
3 Metkovic-1	.12	.14	—	.00	.01	.01	.01	.01	.01	.01	.12	.13	.21	.24	.22	.19	.18	.18	.12	.12
4 Metkovic-2	.11	.13	.01	—	.02	.01	.00	.00	.01	.01	.11	.13	.22	.25	.23	.20	.19	.19	.13	.13
5 Skadarsko Jezero-1	.14	.18	.05	.06	—	.02	.02	.02	.03	.02	.12	.15	.17	.20	.17	.14	.14	.14	.13	.13
6 Skadarsko Jezero-2	.12	.16	.04	.05	.02	—	.01	.01	.02	.01	.11	.14	.16	.20	.17	.14	.13	.13	.12	.12
7 Skopje-1	.08	.11	.04	.03	.07	.06	—	.00	.00	.01	.10	.11	.20	.22	.20	.18	.17	.17	.12	.12
8 Skopje-2	.08	.10	.05	.04	.08	.07	.02	—	.00	.01	.09	.10	.18	.21	.19	.16	.15	.15	.11	.11
9 Igoumenitsa-1	.10	.12	.04	.03	.09	.07	.03	.04	—	.01	.11	.11	.22	.25	.23	.20	.19	.19	.12	.12
10 Igoumenitsa-2	.10	.14	.06	.05	.08	.06	.04	.05	.03	—	.10	.12	.19	.22	.19	.17	.16	.16	.13	.13
<i>ridibunda</i> genomes in hybridogenetic hybrids:																				
11 Zagreb-1	.11	.11	.18	.17	.17	.16	.14	.14	.15	.15	—	.01	.06	.08	.06	.04	.04	.04	.03	.03
12 Zagreb-2	.10	.09	.19	.18	.22	.21	.15	.15	.16	.18	.05	—	.08	.09	.09	.08	.08	.08	.03	.03
13 San Dona-1	.21	.19	.27	.28	.23	.23	.27	.26	.28	.27	.14	.15	—	.01	.01	.01	.02	.02	.08	.08
14 San Dona-2	.23	.21	.29	.30	.25	.25	.29	.28	.30	.29	.12	.13	.02	—	.02	.03	.05	.05	.11	.11
15 Lavello-1	.23	.21	.29	.30	.25	.25	.29	.28	.30	.28	.15	.15	.04	.06	—	.01	.03	.03	.08	.08
16 Lavello-2	.20	.20	.25	.26	.22	.21	.25	.24	.26	.24	.11	.13	.04	.06	.04	—	.00	.00	.06	.06
17 Tarsia-1	.20	.20	.23	.24	.19	.19	.23	.22	.23	.23	.09	.13	.04	.07	.07	.03	—	0	.05	.05
18 Tarsia-2	.20	.20	.23	.24	.19	.19	.23	.22	.23	.23	.09	.13	.04	.07	.07	.03	0	—	.05	.05
19 Catania-1	.16	.15	.16	.17	.18	.18	.18	.18	.17	.20	.08	.08	.11	.13	.13	.09	.07	.07	0	0
20 Catania-2	.16	.15	.16	.17	.18	.18	.18	.18	.17	.20	.08	.08	.11	.13	.13	.09	.07	.07	.07	0

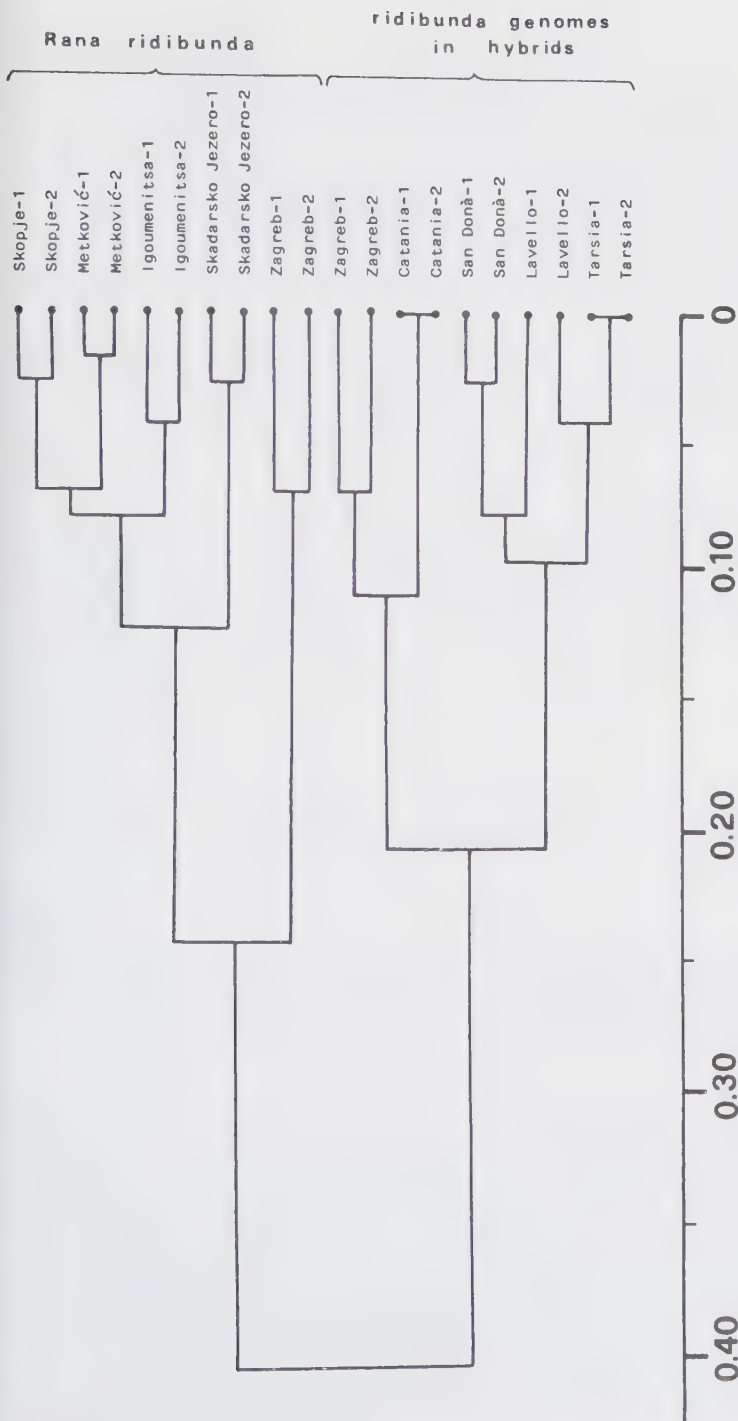


Fig. 2. A phenogram generated by complete linkage cluster analysis (Sneath and Sokal 1973), based on Rogers genetic distances between local samples of Mendelian *Rana ridibunda* populations and of *ridibunda* genomes in hybridogenetic hybrid lineages, using allele frequencies for 19 structural gene loci (A1b omitted). The cophenetic correlation coefficient is 0.88. The absolute genetic distance values obtained for *ridibunda* genomes in a group of hybrids may be numerically inflated due to clonal inheritance subsequent to hybrid synthesis (see text).

tances generally found are those between Rana ridibunda, Zagreb included, and hybrid hemiclones; in the phenogram all hybrid samples join last with the cluster containing all Rana ridibunda populations. The omitted Alb data parallel this pattern, the alleles found in ridibunda genomes of hybrids in Italy occurring rarely, if at all, in Rana ridibunda. (2) A maximum of locally detected clonal diversity in hybrid lineages was found in the peninsular Lavello region instead of in the northern San Donà region close to the Rana ridibunda range (see 3.1). (3) No hemiclones were shared between hybrid samples sympatric with Rana ridibunda and any hybrid samples from Italy, none of the four at Lavello was shared with San Donà, and the Sicilian samples revealed a unique hemiclone, not shared with any mainland Italian region (see 3.1).

These findings of a complex pattern of hemiclone distribution in Italy, while not providing direct estimates for spatial and temporal origin of hybrid lineages, do suggest multiple immigrations of the hemiclinal lineages present in Italy, and they suggest that at least some of these lineages are relatively old.

3.5 THE SUCCESS OF HEMICLONAL HYBRID LINEAGES

The data available for geographical distribution and local frequencies of hybridogenetic hybrids within the Italian region, outside of the present autochthonous Rana ridibunda range, are presented in Table 14 and illustrated in Fig. 3.

Hybridogenetic hybrid lineages are very widespread. They occur from the Po valley drainage area (together with Rana lessonae) throughout the area of the Italian nonhybrid: in the entire Apennine peninsula on either side of the continental divide, and on the island of Sicily. The distances from the present Rana ridibunda range of localities inhabited by hybrids include minima (shortest terrestrial distances through areas below 2000 m altitude) of up to approximately 1400 km (on Sicily, which is separated from mainland Italy by the salt water Strait of Messina).

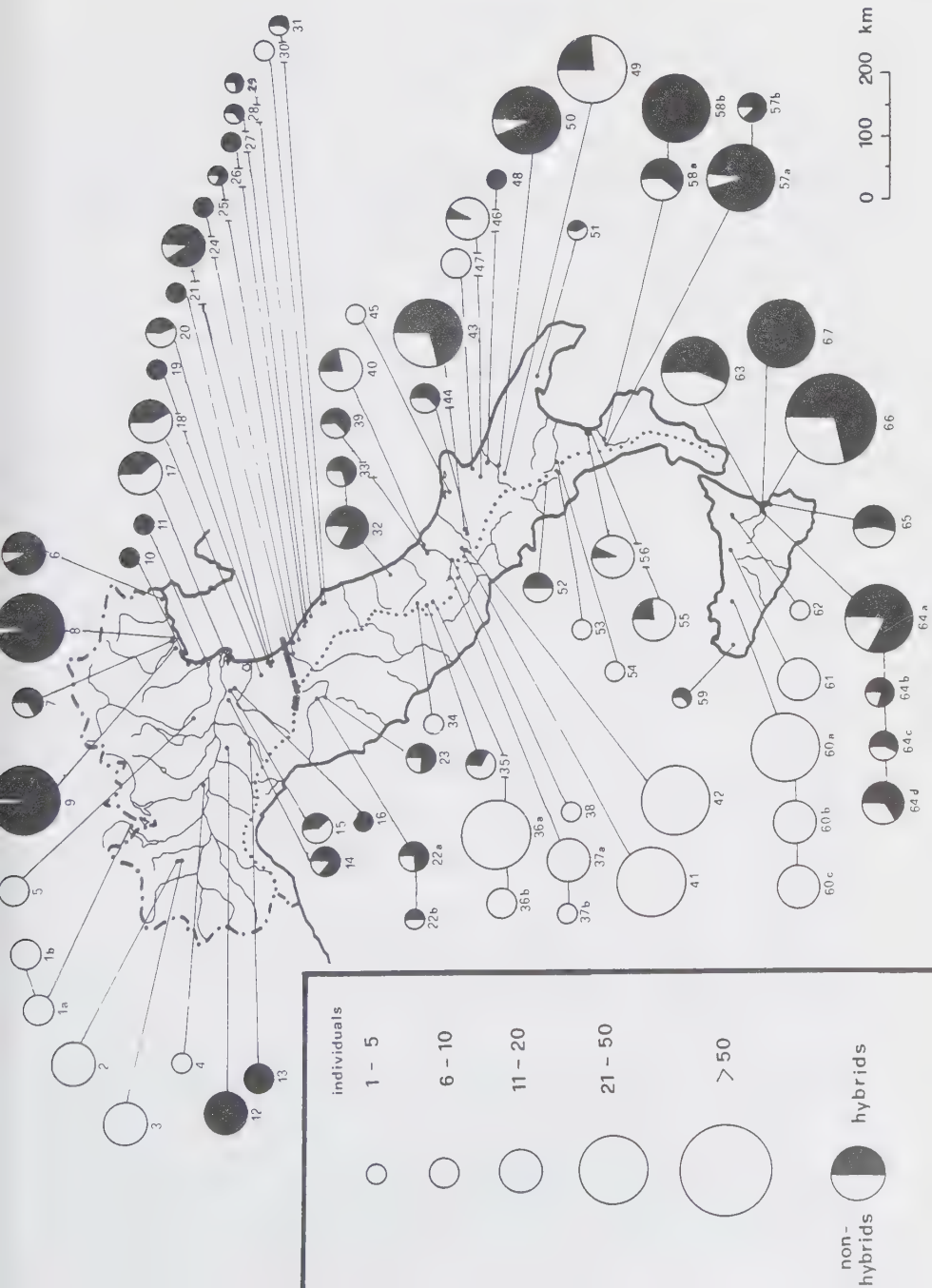
Table 14. Distribution and frequencies of nonhybrids and hybrids in local populations within the Italian region.

All samples consist of postlarval individuals. Taxa were identified by electrophoresis. NH = nonhybrids (*Rana lessonae* or southern nonhybrid); H = hybrids (*Rana esculenta* or southern hybrid). Drainage: A = Adriatic, I = Ionian, T = Tyrrhenian Sea. Localities are keyed to the map (Fig. 3) by numbers and listed in Appendix 1. * = omitted because of incomplete or ambiguous electrophoretic data; ** = 3 individuals (2 NH, 1 H) possibly from this locality are omitted.

Locality	Altitude (m)	Drainage	Month	Sample size			%	
				Total	NH	H	NH	H
1 Magadino	200	A	a Jul	8	8	-	100	0
			b Jun	10	10	-	100	0
2 Buronzo-1	220	A	Sep	20	20	-	100	0
3 Buronzo-2	220	A	Sep	19	19	-	100	0
4 Codogno	60	A	Jul	4	4	-	100	0
5 Verona	20	A	Sep	7	7	-	100	0
6 Isonzo	5	A	Jun	14	1	13	7	93
7 San Donà-0	2	A	Jul	9	3	6	33	67
8 San Donà-1	2	A	Sep	31	1	30	3	97
9 San Donà-2	5	A	Sep	43	1	42	2	98
10 Lugo Venezia	2	A	Jul	2	-	2	0	100
11 Cavarzere	2	A	Jul	5	-	5	0	100
12 Sorbolo	40	A	Jul	12	-	12	0	100
13 Reggio Emilia	± 100	A	Jul	7	-	7	0	100
14 Bondeno	10	A	Jul	6	1	5	17	83
15 Ferrara-1	10	A	Jul	10	6	4	60	40
16 Ferrara-2	5	A	Jul	2 +1*	-	2	0	100
17 Codigoro	2	A	Jul	12	7	5	58	42
18 Lugo	10	A	Jul	14	8	6	57	43
19 Cervia	3	A	Oct	1	-	1	0	100
20 Pisignano	5	A	Oct	7	4	3	57	43
21 Villalta	5	A	Oct	2	-	2	0	100
22 Firenze-Incisa	130	T	a May	8 +1*	2	6	25	75
			b Apr	2 +4*	1	1	50	50
23 Valdarno	140	T	Jul	9	2	7	22	78
24 Santarcangelo	80	A	Oct	19	2	17	11	89
25 Riccione	20	A	Oct	1	-	1	0	100
26 Tombaccia	40	A	Oct	5 +1*	1	4	20	80
27 Pesaro-1	40	A	Oct	1	-	1	0	100
28 Pesaro-2	20	A	Oct	3	1	2	33	67
29 Fano	20	A	Oct	4	1	3	25	75
30 Chiaravalle-1	± 30	A	Mar	2	2	-	100	0
31 Chiaravalle-2	20	A	Oct	5	2	3	40	60
32 Fiume Vomano-1	60	A	Oct	12	2	10	17	83
33 Fiume Vomano-2	50	A	Sep	7	2	5	29	71

Table 14 (continued).

Locality	Altitude (m)	Drainage	Month	Sample size			%	
				Total	NH	H	NH	H
34 Campo Felice	1520	A	Jul	2	2	-	100	0
35 Magliano de' Marsi	730	T	Jul	6	4	2	67	33
36 Ortucchio	680	-	a May	42	42	-	100	0
			b Sep	6	6	-	100	0
37 Pescasseroli	1115	A	a Jul	19	19	-	100	0
			b Apr	4	4	-	100	0
38 Ponte Zittola	820	A	Sep	5	5	-	100	0
39 San Eusanio	180	A	Aug	6	2	4	33	67
40 Val di Sangro	± 40	A	Mar	13	10	3	77	23
41 Lago Civitanova	±1200	A	Sep	33	33	-	100	0
42 Lago di Carpinone	1230	A	Sep	38 **	38	-	100	0**
43 Campobasso-1	510	A	Sep	28	8	20	29	71
44 Campobasso-2	500	A	Oct	8	3	5	38	62
45 Lesina	10	T	Jul	3	3	-	100	0
46 Carapelle	50	A	Mar	13	12	1	92	8
47 Lido di Rivoli	5	A	Sep	10	10	-	100	0
48 Cerignola	100	A	Jul	5	-	5	0	100
49 Lavello-1	200	A	Sep	45	34	11	76	24
50 Lavello-2	130	A	Sep	39	3	36	8	92
51 Taranto	± 50	I	Mar	5	3	2	60	40
52 Sala Consilina	450	I	Jul	8	4	4	50	50
53 Lago Sirino	785	I	Jul	3	3	-	100	0
54 Sinni	650	I	Jul	4	4	-	100	0
55 Villapiana Scalo	< 100	I	Mar	13	10	3	77	23
56 Villapiana Lido	5	I	Jun	15	14	1	93	7
57 Tarsia-1	200	I	a Sep	35	2	33	6	94
			b Jun	8	1	7	13	87
58 Tarsia-2	200	I	a Jun	13	5	8	38	62
			b Sep	29	-	29	0	100
59 Cuddia	150	T	Mar	3	1	2	33	67
60 Bosco di Ficuzza	± 900	T	a Mar	21	21	-	100	0
			b Apr	11	11	-	100	0
			c Jun	14	14	-	100	0
61 Lago Quattrocchi	1030	T	Mar	20	20	-	100	0
62 Saracena	±1000	I	Mar	2	2	-	100	0
63 Piana di Catania-1	10	I	Jun	23	10	13	43	57
64 Piana di Catania-2	10	I	a Apr	39	7	32	18	82
			b Apr	10	2	8	20	80
			c Apr	7	3	4	43	57
			d Apr	11	7	4	64	36
65 Piana di Catania-3	5	I	Jun	13	6	7	46	54
66 Catania-1	5	I	Sep	81	23	58	28	72
67 Catania-2	2	I	Sep	31	-	31	0	100



Locally, the hybridogenetic hybrids are often very abundant and outnumber the syntopic host species, even during spring (Table 14), when a sample bias caused by terrestriality of the non-ridibunda parent (Appendix 5) is not to be expected. Of a total of 1067 water frogs collected in the Italian region, 538 (50.4 %) are hybrids. The hybrid abundance is still more impressive when its absence from Apennine highland regions (Uzzell and Hotz 1979; Table 14) is taken into account: Of a total of 837 water frogs collected at 55 localities in altitudes below 600 m, 536 (64.0 %) are hybrids.

This high success of hybridogenetic hybrid lineages in the Italian region, as reflected in wide geographical distribution and in local abundance, is even more striking if the reproductive capacity of hybrids is considered: All hybrid $\times$ hybrid matings, which are more likely to occur as the hybrid frequency increases at a site, will lead to lethal progeny (Appendix 5).

Fig. 3. Map of Italy showing collecting localities (keyed to the list in Appendix 1 by numbers) for 78 samples of water frogs outside of the present Rana ridibunda range, and indicating local frequencies of hybridogenetic hybrids (Rana esculenta or Italian hybrid) and of the non-ridibunda parental species (Rana lessonae or Italian nonhybrid). The approximate positions of the continental divide (.....) and of the range limits between Rana lessonae (north) and Italian nonhybrid (south) (— — —) are marked.

4 DISCUSSION

Among the Mendelian water frog taxa studied, genic variability was estimated by mean proportion of loci polymorphic per population ($\bar{P}$) and heterozygous per individual ($\bar{H}$) (Tables 6,9). The values obtained were similar to (*Rana ridibunda*, Italian nonhybrid, *Rana lessonae*) or lower than (Yugoslavian and Greek taxon) previously reported variability measures for amphibians based on protein electrophoresis (reviewed by Powell 1975: mean $\bar{H}$ = 10.5 %; Selander 1976: mean $\bar{P}$ = 33.6 %, mean $\bar{H}$ = 8.2 %; Nevo 1978: mean $\bar{P}$ = 26.9 %, mean $\bar{H}$ = 7.9 %; 99 % criterion for P); amphibians usually proved to be more variable than other vertebrates, but less variable than many invertebrate animals and outcrossing vascular plants (Powell 1975, Selander 1976, Nevo 1978, Brown 1979). Specifically, variability estimates for the Mendelian *Rana ridibunda* populations sampled (Tables 5,8) are comparable to those reported for Israel populations of this species (Nevo 1976, Nevo and Yang 1979; using 27 loci, $\bar{P}$ = 38 %, $\bar{H}$ = 2.3 %, average mean number of alleles per locus = 1.46). Genic variability of the Mendelian taxa compared here was highest in *Rana ridibunda*, intermediate in the Italian nonhybrid and in *Rana lessonae*, and lowest in the Greek and the Yugoslavian taxon. A similar decreasing sequence exists in recent range sizes of these taxa: *Rana ridibunda* is the most widespread species, *Rana lessonae* and the Italian nonhybrid have medium-sized ranges, while the two Balkan taxa occupy very restricted geographic areas (Hotz and Uzzell 1982, Tunner and Heppich 1982) and may have undergone bottlenecks of population size in the past glaciation periods (Hotz and Uzzell 1982).

While an extensive body of data on genetic variability is available for natural populations of organisms with Mendelian inheritance (reviews by Lewontin 1974, Nei 1975, Markert 1975, Powell 1975, Selander 1976, Nevo 1978, Brown 1979), knowledge is scarcer for organisms with derived non-recombinational reproduction. Nevertheless, the analyses carried out for clonally reproducing vertebrates (see White 1970, 1978 for invertebrate animals, Brown 1979 for vascular plants) reveal several similarities.

The hemiclonal *Rana* hybrid lineages studied contained a very high amount of individual heterozygosity, between 30 and 50 %, which is much higher than almost all values reported for any Mendelian organisms. This parallels

data obtained for central European Rana esculenta lineages (Uzzell and Berger 1975), and is the overall pattern observed in vertebrates with non-recombinational inheritance, resulting from their interspecies hybrid origin and from their gametogenetic mechanisms (Uzzell 1970), which maintain a fixed heterozygosity level. Such high individual heterozygosity has been reported for diploid hybridogenetic Poeciliopsis hybrids (Schultz 1977; Vrijenhoek, Angus and Schultz 1977: $H = 41-44\%$; Vrijenhoek, Angus and Schultz 1978: $H = 36-50\%$), for parthenogenetic Caucasian Lacerta (Uzzell and Darevsky 1975), and for parthenogenetic Cnemidophorus (diploid lineages: McKinney, Kay and Anderson 1973, $H = 27\%$; Parker and Selander 1976, $\bar{H} = 56\%$; triploid trihybrid lineages: Parker and Selander 1976, $\bar{H} = 71\%$).

The system of clonal reproduction most similar to that in water frogs is known for the group of diploid hybridogenetic all-female fish hybrids of the genus Poeciliopsis living in desert streams of Pacific coastal Mexico, all of which share as one parental species Poeciliopsis monacha and hemiclonally inherit the monacha genome (literature reviewed by Schultz 1977). The amount and pattern of genic diversity in natural populations of such hybridogenetic lineages was studied in two taxa by electrophoresis (Vrijenhoek, Angus and Schultz 1977, 1978) and by histocompatibility experiments (Angus and Schultz 1979, Angus 1980). Clonal diversity at a site was far higher within the range of Poeciliopsis monacha where recurrent hybrid syntheses are possible. This parallels the Rana data reported here, and suggests that in areas of sympatry of both parental species, multiple hybridization is the most significant source of clonal diversity (Vrijenhoek and Schultz 1974; Vrijenhoek, Angus and Schultz 1977, 1978; Angus and Schultz 1979; Angus 1980; present study). The interclonal variability found in these hybridogenetic fish lineages was higher than that previously seen in any other clonal vertebrate: Angus and Schultz (1979) reported 18 hemiclones, 8 of which coexisted at each site; Angus (1980) detected 17 hemiclones among 33 hybrids sympatric with Poeciliopsis monacha, up to 9 coexisting at a site. The present finding of 10 Rana hybrid hemiclones sympatric with Rana ridibunda, 8 at each site (Table 11), join these high values of interclonal diversity. They may even suggest relatively higher diversity in the Rana hybrids, because the estimates are based on electrophoresis: the number of clones detected in Poeciliopsis by tissue grafting was raised, relative to electrophoresis, by factors of 1.7-2.5 in diploid hybridogens (Schultz 1977, Angus and Schultz 1979, Angus 1980) and by a factor of 3 in triploid gynogens (Eisenbrey and Moore 1981).

Muller (1964) proposed that mutations should accumulate more rapidly in organisms without germ line recombination than in recombining organisms, and that, since most mutations are disadvantageous, non-recombining lineages have a ratchet mechanism: their load of deleterious mutations can increase, but not decrease below the level present at any given time in their least loaded clone. Nei (1970, 1975) argued that mutations should accumulate especially rapidly in non-recombined genomes if these are sheltered by other genomes, even if the mutations are not fully recessive and their carriers are at a slight disadvantage. Given that electrophoretic data of the present study and part of the biogeographical data suggest that some hemiclonal hybridogenetic Rana lineages in Italy are relatively old and may even predate the Würm glaciation period, it is surprising that no conclusive electrophoretic evidence for mutations in clonally inherited genomes was found. There is, however, other evidence that mutations have accumulated in these hemiclones in Italy: hybrid $\times$ hybrid matings give lethal progeny. This is demonstrated by the absence of Rana ridibunda individuals among more than a thousand water frogs collected throughout Italy (Table 14). Lethality has been directly documented by crossing experiments (Berger, Uzzell and Hotz 1982): progeny from inter se crosses of hybrids from Sicily uniformly died in an early larval stage. This suggests that recessive deleterious mutants that are sheltered in hybrids by the genome of the non-ridibunda host species have accumulated through Muller's ratchet in non-recombining ridibunda genomes. The data for Italy parallel results obtained in central Europe (Berger 1967, 1971 a, b, 1973 b, 1976, 1979; Blankenhorn, Heusser and Vogel 1971; Heusser and Blankenhorn 1973; Günther 1973; Vogel and Chen 1976; Vogel 1977; Tunner 1979; Binkert, Borner and Chen 1982): inter se crosses of Rana esculenta from the L-E system (Uzzell and Berger 1975; Appendix 5) in most cases gave lethal progeny; there was, however, at least in Poland, less uniformity in offspring abnormalities and in time of death than in the probably much older Sicilian hybrid lineages, and in some cases viable Rana ridibunda progeny were obtained from such crosses. Recessive deleterious mutations in ridibunda genomes of hybrids have been proposed to be a cause of this lethality also (Berger 1976; Graf and Müller 1979; Uzzell 1982 b; Binkert, Borner and Chen 1982).

Hybridogenetic Poeciliopsis lineages also accumulated mutations in their clonally inherited genomes subsequent to hybrid origin. Novel pigmentation patterns and silent electrophoretic alleles were found (Vrijenhoek and Schultz 1974; Vrijenhoek 1975; Vrijenhoek, Angus and Schultz 1977; Leslie and Vri-

jenhoek 1978; Angus 1980). In direct analogy to hybrid $\times$ hybrid lethality in hybridogenetic Rana, an accumulation of deleterious genes in non-recombined genomes of Poeciliopsis hybrids was documented by Leslie and Vrijenhoek (1978), Leslie (1979) and Leslie and Vrijenhoek (1980): They mated hybridogenetic hybrid females to Poeciliopsis monacha and backcrossed the progeny, in which the two monacha genomes recombined, to hybrids. The high mortality of such backcrosses (increased by a factor of about 5 relative to crosses hybrid $\times$ Poeciliopsis monacha) revealed a minimum of 1 to 6 lethal equivalents present in the clonally inherited monacha genomes.

While the data reported here reveal several similarities between the hybridogenetic systems in Rana and in Poeciliopsis, there are also differences. The complex pattern of interclonal variation observed in the widespread hybrid lineages of Rana in Italy, suggesting that some of them are relatively old, is not paralleled by data for Poeciliopsis hybrids. That semiaquatic amphibian hybrids with presumably high terrestrial dispersion capacity occupy larger geographic areas in regions where hybrid syntheses are not possible than do fish hybrids confined to desert streams is not surprising, although the difference is striking (Table 14, Fig. 3; Schultz 1977). Another important difference is the all-femaleness of hybridogenetic Poeciliopsis; this is in marked contrast to the existence of both sexes (Appendix 5) in hybridogenetic Rana lineages. Moore (1976), in a population model, partitioned fitness of hybridogenetic Poeciliopsis lineages and of their syntopic host species into three components: (1) the probability of a female being pregnant, (2) the probability of an offspring being female, and (3) primary fitness. Component (1) was estimated, using data from natural populations, as a function of hybrid/host frequency; (2) amounted to 1 in hybrids, to 0.5 in hosts; and (3) corresponded to fitness in conventional selection models. The results indicated a dichotomy: primary fitness of unisexual hybrids was superior to that of bisexual hosts if $> 85\%$ of the population were unisexuals, and vice versa. Moore concluded that in $> 60\%$ of the range of hybrid lineages the overall distributional success of hybrids was simply attributable to their all-femaleness.

The reported striking success of hybridogenetic Rana lineages far from areas where hybrid syntheses are possible requires a different interpretation, although more female than male progeny is expected for a group of hybrids than for the syntopic host species population, because progeny of host female $\times$ hybrid male matings are all females (Appendix 5). It is pos-

sible that this excess of females plays a role in increasing the hybrid frequency in growing populations in newly colonized habitats, in analogy to Poeciliopsis hybrids (McKay 1971, Moore and McKay 1971, Moore 1975, 1976, Schultz 1977, Angus 1980). Hybrids in Italy are especially frequent, relative to nonhybrids, in disturbed or artificial habitats (Uzzell and Hotz 1979 and unpublished data). On the other hand, the presence of both sexes in hybrids throughout Italy (locally 28-50 %, in sum 39 % of the 264 hybrids studied are males) indicates that such host female $\times$ hybrid male matings are infrequent or have reduced success and that hybrids primarily persist through hybrid female $\times$ host male matings (Appendix 5). Moreover, in populations with high hybrid frequency, common throughout Italy (Table 14), it is presumably difficult for a hybrid to find a nonhybrid mate and there is a high risk of wasting gametes in a lethal hybrid $\times$ hybrid mating. It is tempting to speculate that ridibunda genomes could become adapted not to mate at all if present in a male hybrid rather than to mate with a hybrid female, because the latter results in a higher decrease in average fitness of the locally present ridibunda genomes that are identical by descent for each hemiclone. Natural breeding populations have not been studied in Italy, but some data for central European L-E system Rana esculenta reported by Blankenhorn (1974, 1977) at first glance seem to confirm such a reduced sexual behavior of males; but it is not clear whether the observed patterns reflect an adaptation of the ridibunda genome in hybrid lineages, expressed in a male background, or rather are the result of some ecological and behavioral intermediacy of hybrids between their parental species. However that may be, in the context of these mating systems the frequent predominance of hybrids in local populations strongly suggests that, contrary to Poeciliopsis hybridogens, primary fitness (sensu Moore 1976) accounts for most of the distributional success of hybrid lineages in Italy. This accords with the interpretation of data on reproductive parameters in central European Rana esculenta lineages by Berger and Uzzell (1980), who concluded that primary fitness of hybrids is greater than that of the host species wherever they co-occur.

Other systems of clonal reproduction are known in vertebrates: Gynogenesis (Hubbs and Hubbs 1932), which is matroclinous but requires sperm from a bisexual host species for egg activation, occurs in anamniotes, parthenogenesis in amniotes (Uzzell 1970). Data on genic diversity acquired for gynogenetic and parthenogenetic lineages in natural populations parallel the findings reported here and for other hybridogenetic taxa. Kallman (1962)

detected 3 clones in a population of the diploid gynogenetic interspecific hybrid fish Poecilia formosa by histocompatibility experiments. Among triploid Poeciliopsis hybrids reproducing by gynogenesis, 3 clones were detected in one taxon by electrophoresis, 2 in another (Moore 1977, Schultz 1977); the amount of clonal diversity proved to be much lower than in the congeneric hybridogenetic hybrids, presumably mainly because of the rarity of clone synthesis, which depends on the infrequent production of diploid ova by diploid hybridogenetic hybrids (Schultz 1977). In contrast, Eisenbrey and Moore (1981), using tissue graft analysis, discovered 6 clones, representing 2 electromorph clones, among 24 hybrids of one of these triploid gynogenetic taxa, most of which they interpreted as resulting from mutations subsequent to clone formation. Parker and Selander (1976) analyzed clonal diversity electrophoretically in two parthenogenetic reptile taxa of the genus Cnemidophorus. In triploid trihybrid lineages they found a single clone among 67 individuals from 3 localities; in diploid hybrid lineages 12 clones were detected, 1 to 4 at a site, among 339 individuals from 24 localities. As sources of this diversity, recombination between parental genomes in hybrids was proposed to account for 5 clones, multiple hybridization between the parental species for 4 clones, and mutation subsequent to clone formation for 3 clones.

The number of naturally occurring vertebrate taxa known to reproduce without genetic recombination is steadily increasing (for example Hubbs and Hubbs 1932; Darevsky 1958, 1966, 1967; Miller and Schultz 1959; Maslin 1962, 1971; Uzzell 1963, 1964; Macgregor and Uzzell 1964; Wright and Lowe 1968; Schultz 1969; Hall 1970; Tunner 1973; Uzzell and Darevsky 1975; Graf, Karch and Moreillon 1977; Downs 1978; Uzzell and Hotz 1979; and literature cited in Cole 1975 and in Schultz 1977). There are some general patterns that are common to these clonal vertebrates. (1) They are of interspecies hybrid origin. This has been demonstrated for each well-studied case, and evidence for possible exceptions (Vanzolini 1970, Peters 1971, Bezy 1972) is not conclusive (T. Uzzell, personal communication). (2) They have gametogenetic mechanisms (Uzzell 1970) that lead to meioses without synapsis between chromosomes of different parental species. In most cases studied, a premeiotic germ line endoreduplication of one or all (2 or 3) parental genomes is followed by meiosis with synapsis between sister chromatid derivatives. This has been demonstrated for gynogenetic triploid Ambystoma (Macgregor and Uzzell 1964, Cuellar 1976), for parthenogenetic triploid Cnemidophorus (Cuellar 1971), for gynogenetic triploid Poeciliopsis (Cimino 1972 b) and for hybrido-

genetic diploid Rana (Graf and Müller 1979; Tunner and Heppich 1981; Heppich, Tunner and Greilhuber 1982); see Cimino (1972 a) for hybridogenetic diploid Poeciliopsis. The same gametogenetic mechanism is known for Annelida (Omodeo 1952, 1955; Muldal 1952) and for Orthoptera (White 1980). (3) They have a very high amount of individual heterozygosity, because of (1) and (2). (4) They occur in clusters of low taxonomic rank; no genera or higher categories of clonal vertebrates are known.

That non-recombinational reproduction in vertebrates is coupled with interspecies hybrid origin may be interpreted from two standpoints, which are not exclusive to each other:

(1) The genetic basis for clonal reproduction in vertebrates may only be provided in unique cases of interspecific hybrid genotypes, where specifically distinct genomes, when combined, induce a gametogenesis mechanism that restores nonhaploid euploidy in progeny and that avoids synaptic difficulties during meiosis I due to having different genomes. Such meiotic difficulties of other interspecific hybrids result in their generally low success, because of the reduced fertility of F_1 (or F_2) individuals (Dobzhansky 1951, Mayr 1963, White 1978). The difference is seen in hybrids between Rana ridibunda and either of the two Balkan taxa: these hybrids are not hybridogenetic (Hotz and Uzzell 1983), they occur only in low frequencies, and backcross individuals are very rare (Hotz and Uzzell 1982); this is in marked contrast to the wide distribution and local abundance of those interspecific hybrids of Rana ridibunda that reproduce hemiclonally (Hotz and Uzzell 1983; present study). That clonal reproduction in vertebrates may be a rarely arising phenomenon, happening in cases of specific genomic combinations, is consistent with the observation that clusters of clonal or hemiclonal vertebrate hybrids have a non-random genetic composition, all members of a cluster sharing a single species or a single species group from which one of their parental genomes originates (T. Uzzell in litt. 1982 and personal communication).

(2) Only interspecies hybrids, characterized by maintaining a fixed highly heterozygous quasi- F_1 status without paying the cost of segregational load, may be able, during many generations, to "afford" clonal reproduction with the resulting genetic uniformity among an individual's offspring.

The evolutionary potential of vertebrate taxa reproducing without recombination is not known. Their geographic distribution and local abundance reflects a high momentary success, but their low taxonomic rank suggests a

long-term disadvantage; they have often been regarded as "evolutionary dead ends". Possible causes for short-term success and long-term failure of clonal vertebrates have been extensively discussed (Wright and Lowe 1968; Maslin 1968; White 1970, 1978; Schultz 1971, 1977; Thibault 1974; Uzzell and Darevsky 1975; Williams 1975; Cole 1975, 1978, 1980; Moore 1976; Parker and Selander 1976; Cuellar 1977; Vrijenhoek, Angus and Schultz 1977, 1978; Leslie and Vrijenhoek 1978, 1980; Maynard Smith 1978; Angus and Schultz 1979; Leslie 1979; Bulger and Schultz 1979, 1982; Angus 1980; Uzzell 1982 b). High reproductive capacity because of all-female progeny, heterosis, broader niches and high environmental tolerance, lowered competition pressure by preadaptation to intermediate, relictual or weedy habitats, and preservation of adaptive gene complexes have been proposed to account for the observed success. Genetic inflexibility in generating novel adaptive genotypes and homogeneity between coexisting lineages, both making it difficult to cope with directional selection in changing environments, as well as accumulation of a high load of deleterious mutant genes through Muller's ratchet have been argued to be responsible for a long-term disadvantage. Among the three systems of clonal reproduction occurring in vertebrates, hybridogenesis appears to be the most likely to permit long-term persistence of lineages. It provides the highest amount of genetic variability, as pointed out by Vrijenhoek and Schultz (1974) and Vrijenhoek, Angus and Schultz (1977, 1978), because it increases the number of possible genotypes above the number of locally present clones by the incorporation of different genomes from the plausibly locally adapted gene pool of the syntopic Mendelian host species. Moreover, the clonally inherited genomes are permanently sheltered in a heterozygous condition by host species genomes that are replaced in each generation of hybrid lineages. On the other hand, hybridogenetic lineages seem not to have an independent evolutionary future: they are spatially and temporally constrained to the existence of their host species.

The data for hemiclonal Rana lineages reported here join the increasing body of evidence for a high amount of genetic diversity present in vertebrate taxa that reproduce without recombination. They again illustrate that genomes inherited as completely linked units, when combined in individuals with genomes of another species, in each generation lead to highly successful genotypes characterized by an extreme amount of heterozygosity. The data show that this high genetic heterogeneity within an individual is coupled with reduced interindividual variability in a group of coexisting lineages; but in analogy to other hybridogenetic lineages, interindividual geno-

type diversity among local groups of hybrids occurs, a result of variation in the syntopic host parent's gene pool. The present data are consistent with a relatively great age of some hemiclinal lineages in Italy, far from regions where new hemiclone synthesis is presently possible; they remain candidates for the oldest lineages observed in clonal vertebrates. The findings suggest that continued re-formation of lineages by hybridizations with a steady recruitment of genomes from Mendelian populations (Leslie and Vrijenhoek 1978) is not the only long-term reason for these Rana hemiclones to persist (Uzzell 1982 b). In analogy to a growing body of data for other clonal vertebrates, the findings also suggest that lineages reproducing without recombination are less severely limited in their potential to persist than has often been assumed. The wide distribution and local abundance of hybridogenetic lineages in Italy reported here and their possibly great age once again illustrate the high success of a vertebrate reproducing without recombination. More importantly, however, this success is not coupled with all-female-ness as in other clonal vertebrates: hybridogenetic Rana lineages do not have a two-fold advantage in reproductive capacity. This suggests that heterosis of individual hybrids, reflected as viability or as ecological plasticity, plays a major role in the high success of hemiclinal lineages. Uzzell (1982 b) pointed out that Rana esculenta in central Europe, in comparison to the parental species, appears to be relatively well adapted, both in survival (growth rate, vitality, resistance to lack of oxygen: Berger 1971 b, Heusser and Blankenhorn 1973, Berger and Uzzell 1977, Tunner and Nopp 1979) and in reproductive parameters (Berger and Uzzell 1980). Somatic vigor has also been found in hybridogenetic Poeciliopsis hybrids (Schultz 1971, Moore 1976, Bulger and Schultz 1979, 1982). The high success here reported for hemiclonally reproducing non-all-female lineages, consisting of highly heterozygous hybrids, does not parallel the relatively low importance of heterosis for the success of hemiclinal lineages proposed by Moore (1976) for Poeciliopsis hybrids. The pattern suggests that individual heterosis arising from a diverse genomic combination that is permanently replicated in hybrid lineages may well be one of the major sources of the general success of other non-recombinational vertebrate lineages also.

5 SUMMARY

1. Electrophoretically demonstrable minima of genic variation were determined for proteins encoded by 20 structural gene loci in postlarval samples of European water frogs to examine effects of Mendelian and non-recombinational reproduction on their genetic structure. 352 frogs with Mendelian inheritance representing 5 taxa (Rana ridibunda, R. lessonae, Italian nonhybrid, Yugoslavian taxon, Greek taxon) were compared with 304 hybridogenetic interspecific hybrids that hemiclonally inherit one parental genome (of R. ridibunda) and in gametogenesis exclude the other parental genome (of syntopic R. lessonae or Italian nonhybrid). Two replicate samples each from 5 Balkan regions (R. ridibunda range) and from 4 Italian regions (outside of R. ridibunda range) were used. Hybrids were contained in samples from one Balkan and from all Italian regions.
2. Ploidy was determined by erythrocyte size and electrophoretic phenotypes: only 2 hybrids were probably triploid, one from southern Italy (1 ridibunda genome, 2 Italian nonhybrid genomes), one from the Balkans (2 ridibunda genomes, 1 lessonae genome).
3. Hybridogenetic gametogenesis was confirmed for hybrids from each region by electrophoretic comparison of somatic tissues and germ line cells.
4. Genic variability in Mendelian taxa, estimated by proportion of polymorphic loci, number of alleles and individual heterozygosity, was highest in R. ridibunda, intermediate in the Italian nonhybrid and R. lessonae, lowest in the Greek and the Yugoslavian taxon. Values were average or low (Yugoslavian and Greek taxon) compared to other amphibians. R. ridibunda was more variable in the inland Balkans than in coastal areas, Italian nonhybrids were more variable on the mainland than on Sicily.
5. Hybridogenetic hybrids were highly heterozygous ($H = 30-50\%$), differing from R. ridibunda by a factor of up to 6.6, and by more from the other Mendelian taxa. This heterozygosity reflects the genetic difference between the two parental genomes in these hybrids.
6. Clonal diversity among ridibunda genomes in coexisting hybrid lineages was restricted compared to Mendelian R. ridibunda populations.
7. Clonal diversity at a site was highest in sympatry of hybrids with R. ridibunda (10 hemiclones in region, 8 at each site). This is explained by multiple hybridizations between parental species. R. ridibunda and ridibunda genomes of hybrids had significantly different allele frequencies at 2 loci.

8. Hybrids allopatric to R. ridibunda had reduced clonal diversity (1-4 hemiclones per site). No continuous decrease of clonal diversity in hybrid samples occurred with increasing distance from the R. ridibunda range; a maximum was observed for southern central Italy.
9. In Italy 7 hemiclones were detected, none of which was shared with hybrids sympatric with R. ridibunda. No hemiclones were shared between the Po valley and peninsular Italy, and Sicily had a single, unique hemi-clone. This pattern, the clonal diversity at the sites, and an allele not known in R. ridibunda occurring in 2 different southern Italian hemi- clones, suggest multiple immigration and a relatively great age of some hybrid lineages, rather than an exclusive recent immigration from the present R. ridibunda range.
10. No electrophoretic evidence for mutations in clonally inherited genomes subsequent to clone formation was found, but lethality of matings between hybrids suggests accumulation of deleterious mutant genes.
11. Hybridogenetic hybrid lineages are highly successful in Italy, allopatric to R. ridibunda, as judged by using 689 additional frogs in 70 samples: they are widespread (Po valley, throughout peninsular Italy and Sicily, up to at least 1400 km from the R. ridibunda range) and locally abundant (50 % of all frogs, 64 % of frogs from lowlands).
12. Contrary to other clonal vertebrates, hybridogenetic Rana lineages are not all-female. This suggests that individual heterosis is a major source for the high success of possibly old lineages far from regions where clone formation is possible.

APPENDIX 1

LOCALITY LIST

The replicate samples used for the present study were collected at the following localities:

Balkan region (within the present autochthonous Rana ridibunda range):

Zagreb-1: Yugoslavia, Hrvatska. Jastrebarsko, 30 km WSW Zagreb. Stream at the southern edge of the village, at road nr. 7 Zagreb - Karlovac. Collected 7.8.1980 (with M. Frauenfelder; field numbers = FN 397-431) and 27.9.1980 (with T. Uzzell; FN 735-737).

Zagreb-2: Yugoslavia, Hrvatska. Stream N of Kupinec at road Klinca Selo - Pisarovina, 20 km SW Zagreb. 10 km E of Zagreb-1. 7.8.1980 (with M. Frauenfelder; FN 432-467) and 26.9.1980 (with T. Uzzell, FN 738-740).

Metković-1: Yugoslavia, Dalmacija. Ditch 3 km N Metković, 1 km SE Vid, on the right side of the river Neretva. 1. and 5.8.1980 (with M. Frauenfelder; FN 283-308).

Metković-2: Yugoslavia, Dalmacija. Ditches 2 km SW Metković, at road nr. 10 Metković - Dubrovnik, on the left side of the river Neretva. 5 km S of Metković-1. 2.8.1980 (with M. Frauenfelder; FN 309-334).

Skadarsko Jezero-1: Yugoslavia, Crna Gora. Canal to the lake at Virpazar, W edge of Skadarsko Jezero. 3.8.1980 (with M. Frauenfelder; FN 335-367) and 11.9.1980 (with T. Uzzell; FN 479-484, 489-491).

Skadarsko Jezero-2: Yugoslavia, Crna Gora. Canal to the lake at Vranjina, NW edge of Skadarsko Jezero, at road E 27 Kotor - Titograd. 7 km NE of Skadarsko Jezero-1. 3.8.1980 (with M. Frauenfelder; FN 368-396).

Skopje-1: Yugoslavia, Makedonija. Stream 10 km SE Skopje, at road nr. 1 Skopje - Thessaloniki. 13.9.1980 (with T. Uzzell; FN 524-548).

Skopje-2: Yugoslavia, Makedonija. Stream 15 km SE Skopje, SW of road nr. 1 Skopje - Thessaloniki. About 5 km SE of Skopje-1. 13.9.1980 (with T. Uzzell; FN 549-589).

Igoumenitsa-1: Greece, Epeiros. Ditch 4 km N Igoumenitsa, Kalamas delta region, on the left side of the river Kalamas. 15. and 16.9.1980 (with T. Uzzell; FN 590-622).

Igoumenitsa-2: Greece, Epeiros. Canal 10 km NNW Igoumenitsa, Kalamas delta region, on the right side of the river Kalamas. About 6 km NW of Igoumenitsa-1. 17.9.1980 (with T. Uzzell; FN 623-653).

Italian region (outside the present autochthonous Rana ridibunda range):

San Donà-1: Italy, Veneto. Ditch at Isiata, 4 km SE San Donà di Piave, on the left side of the Fiume Piave, 30 km NE Venezia. 25.9.1980 (with T. Uzzell; FN 920-950).

San Donà-2: Italy, Veneto. Stream between Pralongo and Fornaci, 6 km W Fossalta di Piave, 10 km WNW San Donà di Piave, on the right side of the Fiume Piave, 25 km NE Venezia. 14 km WNW of San Donà-1. 27.9.1980 (with T. Uzzell; FN 951-993).

Lavello-1: Italy, Basilicata. Stream S of the Lago di Abate Alonia, 6 km SW Lavello, 40 km N Potenza. 22. and 23.9.1980 (with T. Uzzell; FN 836-880).

Lavello-2: Italy, Basilicata. Canal in the Fiume Ofanto valley, NW of the road SS 93, about 8 km NE Lavello, 50 km N Potenza. About 12 km NE of Lavello-1. 23.9.1980 (with T. Uzzell; FN 881-919).

Tarsia-1: Italy, Calabria. Canal W of the Fiume Crati, 2 km SW Tarsia at road nr. 19, 30 km N Cosenza. 21.9.1980 (with T. Uzzell; FN 772-806).

Tarsia-2: Italy, Calabria. Shore of the reservoir of the Fiume Crati, about 1 km SE Tarsia, 35 km N Cosenza. About 4 km NE of Tarsia-1. 21.9.1980 (with T. Uzzell; FN 807-835).

Catania-1: Italy, Sicilia. Stream 8 km S Catania, at road SS 114, 4 km N of the Fiume Simeto bridge. 19.9.1980 (with T. Uzzell; FN 654-734).

Catania-2: Italy, Sicilia. Canal at Lido di Plaia at road SS 114, shore region at southern town edge of Catania. 5 km N of Catania-1. 20.9.1980 (with T. Uzzell; FN 741-771).

Additional samples from the Italian region were collected at the following localities (keyed to the map in Fig. 3, p. 51, by numbers):

Switzerland:

1 Magadino: Canton Ticino, woodland pools on the left side of the Ticino river, 1 km NE Magadino, 12 km WSW Bellinzona. (a) July 1974 (8 individuals; collected with C. Spolsky and T. Uzzell) and (b) 11.6.1981 (10; with T. Uzzell).

Italy:

- 2 Buronzo-1: Piemonte, rice fields and ditches 7.4 km N Buronzo, 32 km WNW Novara. 8.9.1978 (20).
- 3 Buronzo-2: Piemonte, ditches and meadows 5.8 km N Buronzo, 31 km WNW Novara. 8.9.1978 (19).
- 4 Codogno: Lombardia, ditch at Cavacurta, fiume Adda region, 4 km NE Codogno, 16 km N Piacenza. 16.7.1979 (4; with T. Uzzell).
- 5 Verona: Veneto, irrigation ditch at Bovolone, NE village end at road to Oppeano, 23 km SSE Verona. 22.9.1978 (7).
- 6 Isonzo: Friuli-Venezia Giulia, drainage ditch 2 km S of Fiume d'Isonzo on road from Grado to Monfalcone, 28 km WNW Trieste. 15.6.1974 (14; by E. Sochurek, C. Spolsky and T. Uzzell).
- 7 San Donà-0: Veneto, drainage canal 5 km SE San Donà di Piave, 30 km NE Venezia. 14.7.1979 (9; with T. Uzzell).
- 8 = San Donà-1 of the present study.
- 9 = San Donà-2 of the present study.
- 10 Lugo Venezia: Veneto, large canal at Lugo Venezia, at road SS 309, 17 km SW Venezia. 14.7.1979 (2; with T. Uzzell).
- 11 Cavarzere: Veneto, canal and ditches between Cavarzere and Chioggia, 34 km SSW Venezia. 14.7.1979 (5; with T. Uzzell).
- 12 Sorbolo: Emilia-Romagna, ditch between Sorbolo and Casaltone, Torrente Enza region, 10 km WNW Parma. 16.7.1979 (12; with T. Uzzell).
- 13 Reggio Emilia: Emilia-Romagna, river between Reggio nell'Emilia and Vezzano, about 10 km SSW Reggio. 15.7.1979 (with T. Uzzell).
- 14 Bondeno: Emilia-Romagna, ditch 4 km E Bondeno, S of the Po, 12 km NW Ferrara. 15.7.1979 (6; with T. Uzzell).
- 15 Ferrara-1: Emilia-Romagna, large ditch S Pontelagoscuro, S of the Po, NW edge of Ferrara. 15.7.1979 (10; with T. Uzzell).
- 16 Ferrara-2: Emilia-Romagna, ditch 6 km S Ferrara, by Autostrada entrance. 15.7.1979 (3; with T. Uzzell).
- 17 Codigoro: Emilia-Romagna, large canal between Codigoro and Pomposa, Po delta region, 41 km E Ferrara. 15.7.1979 (12; with T. Uzzell).
- 18 Lugo: Emilia-Romagna, small drainage canal at W edge of Lugo, 24 km W Ravenna. 15.7.1979 (14; with T. Uzzell).
- 19 Cervia: Emilia-Romagna, ditch between Madonna della Neve and Villa Inferno, 3 km SW Cervia, 24 km E Forlì. 16.10.1978 (1; with S. Bruno).

- 20 Pisignano: Emilia-Romagna, canal and rain pools 4 km SE Pisignano, 7 km SW Cervia, 21 km E Forlì. 6.10.1979 (7; with T. Uzzell).
- 21 Villalta Cesenatico: Emilia-Romagna, canal Fossa Montaletto along Via S. Pellegrina between Pisignano and Villalta, 25 km E Forlì. 16.10.1978 (2; with S. Bruno).
- 22 Firenze-Incisa: Toscana, gravel pit area about 7 km S of A1 exit Firenze-Incisa, Arno valley E of Autostrada, 26 km SE Firenze. (a) 5.5.1978 (9; by C. Spolsky and T. Uzzell) and (b) 17.4.1980 (6).
- 23 Valdarno: Toscana, pools 2 km S San Giovanni Valdarno, Arno valley, 34 km SE Firenze. July 1974 (9; by C. Spolsky and T. Uzzell).
- 24 Santarcangelo: Emilia-Romagna, ponds and ditches in a gravel pit area of the Fiume Marecchia region 1 km S Poggio Berni, 6 km SSW Santarcangelo di Romagna, 10 km NNW San Marino. 6.10.1979 (19; with T. Uzzell).
- 25 Riccione: Emilia-Romagna, water hole near a pond 3 km SW Riccione, near A14 exit, 10 km SE Rimini. 5.10.1979 (1; with T. Uzzell).
- 26 Tombaccia: Emilia-Romagna, ponds 1-2 km SW Tombaccia in the Torrente Conca valley, 7 km SW Cattolica, 20 km W Pesaro. 6.10.1979 (6; with T. Uzzell).
- 27 Pesaro-1: Marche, river shore of the Fiume Foglia 12 km SW Pesaro, between Montelabbate and Osteria Nuova. 7.10.1979 (1; with T. Uzzell).
- 28 Pesaro-2: Marche, pond in old gravel pit area at Villa Fastiggi, Fiume Foglia valley, 5 km SW Pesaro. 7.10.1979 (3; with T. Uzzell).
- 29 Fano: Marche, artificial pond near Autostrada A14 in the Fiume Metauro valley, 4 km S Fano, 13 km SE Pesaro. 7.10.1979 (4; with T. Uzzell).
- 30 Chiaravalle-1: Marche, pond in gravel pit area near Chiaravalle, about 16 km W Ancona. 24.3.1977 (2; with T. Uzzell).
- 31 Chiaravalle-2: Marche, pools and ditches near the shore of the Fiume Esino S Chiaravalle, 16 km WSW Ancona. 16.10.1978 (5; with S. Bruno).
- 32 Fiume Vomano-1: Abruzzo, ditch and concrete basin near the Fiume Vomano, between Fontanelle and Faiete, 32 km NW Pescara. 15.10.1978 (12; with S. Bruno).
- 33 Fiume Vomano-2: Abruzzo, canal and basin in the Fiume Vomano valley, 2 km WNW Fontanelle, about 12 km WSW the Fiume Vomano mouth, 32 km NW Pescara. 27.9.1979 (7; with T. Uzzell).
- 34 Campo Felice: Abruzzo, pools on the high plain Piano di Campo Felice, Monte Velino region, 15 km S L'Aquila. 2.7.1974 (2; by C. Spolsky and T. Uzzell).

- 35 Magliano de' Marsi: Abruzzo, irrigation canal near Magliano de' Marsi, 10 km NW Avezzano. 3.7.1974 (6; by C. Spolsky and T. Uzzell).
- 36 Ortucchio: Abruzzo, pond in Ortucchio, 19 km SE Avezzano. (a) 6.5.1978 (42; by C. Spolsky and T. Uzzell) and (b) 24.9.1979 (6; with T. Uzzell).
- 37 Pescasseroli: Abruzzo, stream and ponds along the Fiume Sangro, Piana di Opi, 3 km SE Pescasseroli. (a) 2.7.1974 (19; by C. Spolsky and T. Uzzell) and (b) 8.4.1977 (4; with T. Uzzell).
- 38 Ponte Zittola: Abruzzo, stream at Ponte Zittola, 4 km SSW Castel di Sangro, 20 km NW Isernia. 30.9.1979 (5; with T. Uzzell).
- 39 San Eusanio del Sangro: Abruzzo, pond near San Eusanio del Sangro, Laghetto del Medico, 8 km SW Lanciano, 25 km SE Chieti. 16.8.1978 (6; by L. Vitelli).
- 40 Val di Sangro: Abruzzo, artificial concrete basin in the Sangro valley, about 8 km SW of the Sangro mouth, 30 km SE Chieti. 25.3.1977 (13; with T. Uzzell).
- 41 Lago Civitanova: Molise, residual water hole of a temporary lake and adjacent artificial basin on the high plateau between Frosolone, Carpinone and Civitanova, 16 km E Isernia. 26. and 30.9.1979 (33; with S. Bruno and T. Uzzell).
- 42 Lago di Carpinone: Molise, lake Lago di Carpinone 4 km NE Carpinone, on the high plateau between Frosolone, Carpinone and Civitanova, 12 km E Isernia. 30.9.1979 (38; with T. Uzzell).
- 43 Campobasso-1: Molise, artificial pond about 3 km SW Vinchiaturro, near the road SS 87, 13 km SW Campobasso. 26. and 30.9.1979 (28; with S. Bruno and T. Uzzell).
- 44 Campobasso-2: Molise, river Torrente Quirino at road SS 17, 4 km SW Vinchiaturro, 13 km SW Campobasso. 1.10.1981 (8; with M. Jozefiak).
- 45 Lesina: Puglia, ditch 1 km SE Lesina 44 km NNW Foggia. 2.7.1974 (3; by C. Spolsky and T. Uzzell).
- 46 Carapelle: Puglia, stream Torrente Carapelle at Autostrada A 14, 18 km NW Cerignola, 20 km SE Foggia. 25.3.1977 (13; with T. Uzzell).
- 47 Lido di Rivoli: Puglia, canal at road SS 545 between Masseteria San Vito and Lido di Rivoli, 22 km N Cerignola, 31 km E Foggia. 22.9.1980 (10; with T. Uzzell).
- 48 Cerignola: Puglia, storm drain 6 km E Cerignola, N of the Autostrada intersection A 14 and A 17. 1.7.1974 (5; by C. Spolsky and T. Uzzell).

49 = Lavello-1 of the present study.

50 = Lavello-2 of the present study.

- 51 Taranto: Puglia, stream between Grottaglie and Taranto, 10 km NE Taranto. 25.3.1977 (5; with T. Uzzell).
- 52 Sala Consilina: Campania, ditch 2 km W Sala Consilina, Tanagro valley. 1.7.1974 (8; by C. Spolsky and T. Uzzell).
- 53 Lago Sirino: Basilicata, lake near road nr. 19 on southern slope of Monte Sirino, 5 km SE Lagonegro. 1.7.1974 (3; by C. Spolsky and T. Uzzell).
- 54 Sinni: Basilicata, lake in Fiume Sinni, between Pecorone and Latronico, about 64 km SSE Potenza. 1.7.1974 (4; by C. Spolsky and T. Uzzell).
- 55 Villapiana Scalo: Calabria, irrigation ditch near Torrente Raganello, about 5 km S Villapiana Scalo, about 22 km E Castrovillari. 25.3.1977 (13; with T. Uzzell).
- 56 Villapiana Lido: Calabria, ditch at Villapiana Lido, W of the road SS 106, 25 km E Castrovillari. 11.6.1982 (15; with T. Uzzell).
- 57 = Tarsia-1 of the present study (a), and (b) 11.6.1982 (8; with T. Uzzell).
- 58 = Tarsia-2 of the present study (b), and (a) 30.6.1974 (13; by C. Spolsky and T. Uzzell).
- 59 Cuddia: Sicilia, river Fiume Cuddia below Lago di Rubino, at Ponte della Collura, 11 km NW Salemi, 22 km SE Trapani. 28.3.1977 (3; with T. Uzzell).
- 60 Bosco di Ficuzza: Sicilia, pond near Godrano in Bosco di Ficuzza, about 25 km SSE Palermo. (a) 27.3.1977 (21; with T. Uzzell), (b) 28.4.1978 (11) and (c) 10.6.1982 (14; with T. Uzzell).
- 61 Lago Quattrocchi: Sicilia, pond Lago Quattrocchi, 4 km SE Mistretta. 27.3.1977 (20; with T. Uzzell).
- 62 Torrente di Saracena: Sicilia, stream in the Torrente di Saracena region, 10 km W Randazzo. 26.3.1977 (2; with T. Uzzell).
- 63 Piana di Catania-1: Sicilia, irrigation ditches and drainage canals in the Piana di Catania, between Fiume Gornalunga and Fiume Simeto, about 16 km SSW Catania. 30.6.1974 (23; by C. Spolsky and T. Uzzell).
- 64 Piana di Catania-2: Sicilia, irrigation ditches, canals, basins and loamy pools in the Piana di Catania near the Fiume Simeto, about 13 km SW Catania. (a) 7.4.1977 (39; with T. Uzzell), (b) 3. and 4.4.1978 (10; with M. Frauenfelder), (c) 29.4.1978 (7), and (d) April 1979 (11).

65 Piana di Catania-3: Sicilia, canal along the road SS 114, 4 km S of the Fiume Simeto mouth, 15 km S Catania. 10.6.1982 (13; with T. Uzzell).

66 = Catania-1 of the present study.

67 = Catania-2 of the present study.

Localities 23,34,35,37,45,48,52,53,54,58 and 63 were reported by Uzzell and Hotz (1979).

APPENDIX 2

ELECTROPHORETIC PHENOTYPES AND ALLELE DISTRIBUTIONS

Only electrophoretic patterns observed in the replicate samples used for this study are here reported. For each locus studied, the electrophoretic phenotypes observed in somatic tissues (skeletal and heart muscle, liver, kidney; plasma) and their genetic interpretations are described; the taxonomic and geographic distribution of the electrophoretically distinguished alleles is summarized for the samples used to facilitate comparisons between taxa; the overall distribution of these alleles among western Palearctic water frogs is briefly noted when known; and previously used allele names are reported as synonyms. For multilocus enzyme systems (AAT, IDH, LDH, MDH, Pep, PGM) numbers are assigned to loci in sequence of decreasing anodal mobility of the allelic product most common in Rana ridibunda; numbers are also given to loci encoding for non-specifically stained proteins (MProt) in sequence of decreasing anodal mobility of their products. Allelic variants at a locus are named by assigning them letters alphabetically (Hotz and Uzzell 1982); except for several alleles that have already been named (Hotz and Uzzell 1982, 1983), the letter assignment is in sequence of decreasing anodal mobility. Some letters not included refer to alleles occurring in water frogs from other parts of the western Palearctic area (Hotz and Uzzell, in preparation). At any locus, electrophoretically indistinguishable translation products are treated as though encoded by the same allele; repeatably distinguishable translation products are assumed to represent different alleles rather than posttranslational modifications. For the Alb locus, two electrophoretic variants with barely distinguishable mobilities (b of Rana ridibunda and g of the Italian nonhybrid) have been shown by immunology to represent different alleles (Uzzell in press). Taxa were identified by previously reported electrophoretic phenotypes of some diagnostic loci that parallel morphological data (Uzzell and Hotz 1979 for Italy; Hotz and Uzzell 1982 for the Balkans). Electrophoretic variation reported for Rana lessonae and the Italian nonhybrid is at least partly based on the non-ridibunda genomes of hybrids; see Appendix 4 for the assignment of electrophoretic alleles of heterozygous hybrids to parental genomes.

AAT-1

Electrophoretic patterns. Two AAT loci, both variable in European water frogs, are active in skeletal and heart muscle, liver and kidney (see Uzzell and Berger 1975; Graf, Karch and Moreillon 1977). Both on low and high pH tris-citrate gels the allelic products of the two loci overlapped extensively, and for this reason only the AAT-1 locus was scorable in all individuals. AAT-1 accounted for relatively higher activity in skeletal and heart muscle and in kidney samples, whereas in liver samples the two loci led to equally intense bands. For AAT-1, 13 different phenotypes were observed; all patterns are consistent with the assumption that 10 different alleles (a, b, c, d, e, f, g, h, i, j) code for the subunits of a dimeric enzyme. Dense single bands without satellite bands occurred in three mobilities; they apparently represent homozygous patterns (a/a, e/e, g/g). Ten different 3-banded patterns, consisting of two weak bands plus a more intense intermediate band midway between them, apparently represent heterozygous patterns. The two less intense homodimer bands of such heterozygote phenotypes either had the same position as two of the single bands observed (2 kinds of heterozygotes: a/e, e/g), or one of them had a mobility not observed as a single band (7 kinds: b/e, c/e, d/e, e/f, e/h, e/i, e/j), or both of them were in one of these positions not observed as single bands (1 kind: d/j). On low pH tris-citrate gels, electromorphs a, b, c, d and usually e (close to the origin) moved towards the anode, the others towards the cathode; on high pH tris-citrate gels all electromorphs had anodal mobility. A faint band approximately in the g position observed in many e/e individuals is apparently due to the AAT-2 locus. When heterozygous, the AAT-2 locus also yielded 3-banded dimer patterns (see also Graf, Karch and Moreillon 1977), but no interlocus heterodimer patterns were observed.

Allele distribution among samples used. The e allele is the most abundant: it is the most common allele of Rana ridibunda (monomorphic at Zagreb-1, Skadarsko Jezero and Igoumenitsa-1 and predominant in all other samples), is the only allele found in the Yugoslavian taxon, vastly predominates in the Greek taxon, and is the most common allele of the Italian nonhybrid (monomorphic at Catania, predominant at Lavello and Tarsia); it also occurs at low frequencies in Rana lessonae from San Donà (judged by some Rana esculenta yielding only the e electromorph, interpreted as e/e homozygotes). This e allele is also the allele in ridibunda genomes of all hybrids from Zagreb, San Donà, Tarsia and Catania, and in many hybrids from Lavello. The a allele

occurs at moderate to low frequencies in the Italian nonhybrid at Lavello. The b allele was found in one Rana ridibunda from Metković-2 and at low frequency in the Italian nonhybrid genomes of hybrids from Tarsia-2. Whether this mobility (indistinguishable on pH 5.9 and 7.0 tris-citrate gels), which is found at very low frequency in two different taxa from two distant areas, reflects allelic identity or convergence, is unknown. The c allele is widespread at low frequencies in Rana ridibunda (Zagreb, Metković, Skopje). The d allele was found at low to moderate frequency in the Italian nonhybrid (Lavello, Tarsia). Rana ridibunda from Skopje and Igoumenitsa had the allele f in low frequencies; the same allele was found in one individual of the Greek taxon from Igoumenitsa (it cannot be excluded that this individual represents a backcross, to Rana ridibunda, of a Rana ridibunda × Greek taxon hybrid; see Hotz and Uzzell 1982). The g allele is typical of Rana lessonae (monomorphic at Zagreb, predominant at San Donà). The h allele was observed as a rare Italian nonhybrid allele in a hybrid from Tarsia; the i allele as a rare lessonae allele in a hybrid from San Donà. The j allele represents one of two ridibunda alleles occurring in hybrid lineages from Lavello (see Appendix 4).

General allele distribution. The e allele characterizes Rana ridibunda also in central and eastern Europe (Uzzell and Berger 1975; Graf, Karch and Moreillon 1977; Uzzell, Hotz and Berger 1980; Tunner 1980; unpublished data), is present at low frequencies in Rana lessonae throughout the Po valley (unpublished) and in Poland (Uzzell and Berger 1975; possibly introgressed from Rana ridibunda, see Uzzell 1982 b), is the most common Italian nonhybrid allele throughout the peninsula and on Sicily (unpublished data), is the ridibunda allele in Rana ridibunda × Rana perezi hybrid lineages in southern France and also occurs in Rana perezi (Graf, Karch and Moreillon 1977; unpublished data). The g allele characterizes Rana lessonae throughout central Europe and northern Italy (Uzzell and Berger 1975; Graf, Karch and Moreillon 1977; Uzzell, Hotz and Berger 1980; Tunner 1980; unpublished data); it occurs in medium to low frequencies in the Italian nonhybrid in the northern to central Adriatic coastal region (unpublished), was rarely observed in central European Rana ridibunda, possibly due to introgression (Uzzell and Berger 1975; see Uzzell, Günther and Berger 1977; Uzzell 1982 b), and has been reported to occur in Rana perezi (Graf, Karch and Moreillon 1977; this might however be a different allele: unpublished data). The j allele, so far exclusively found in ridibunda genomes of the Italian hybrid, probably is widespread on the Adriatic side of central and southern peninsu-

lar Italy (Uzzell and Hotz unpublished). The a, b, c, d, f, h and i alleles have not yet been reported elsewhere for water frogs.

Synonyms. e: S (Uzzell and Berger 1975), 100 (Graf, Karch and Moreillon 1977), 100 (Tunner 1980);

g: F (Uzzell and Berger 1975), 26 (Graf, Karch and Moreillon 1977), 94 (Tunner 1980);

a, b, c, d, f, h, i, j: none (b or c perhaps 147: Graf, Karch and Moreillon 1977).

AK

Electrophoretic patterns. No variation was detected at this locus, scored in skeletal muscle and, with less activity, in kidney samples. A single, uniform, distinct, rather broad band of anodal mobility, without subbands, was observed on low pH tris-citrate gels for all individuals. This is interpreted as representing a single allele (a). AK was revealed as a faint band also by staining for CK.

Allele distribution among samples used. The a allele of this monomorphic locus is shared by all individuals of each taxon.

General allele distribution. The a allele occurs in central European Rana lessonae (Tunner 1980); it was also found in Rana perezi and in Rana saharica (unpublished data).

Synonyms. a: 100 (Tunner 1980).

Ald

Electrophoretic patterns. This locus was found active only in skeletal muscle tissues. At least three different phenotypes, moving anodally, were distinguished on high pH tris-citrate gels. They are apparently due to at least two different alleles (a, b). Intense sharp single bands, without subbands, occurred in two different mobilities; the more anodal representing a/a, the less anodal, b/b homozygote patterns. Broad smears, more intense in the center and ending with reduced intensity at the a and b positions apparently reflect a/b heterozygote patterns of a non-monomeric enzyme.

Since some individuals yielded different, unclear smeary patterns with uncertain interpretations, this locus is not included in the main body of this study.

Allele distribution among samples used. The a allele is typical of Rana lessona and the Italian nonhybrid; the b allele characterizes Rana ridibunda, the Yugoslavian and the Greek taxon, and occurs as the ridibunda allele in hybridogenetic hybrid lineages from all regions.

General allele distribution. Not yet established.

Synonyms. None.

CK

Electrophoretic patterns. Three different phenotypes were observed in skeletal muscle tissues, consistent with there being two alleles (a, b) coding for the subunits of a dimeric enzyme. On low pH tris-citrate gels, very intense single bands, moving anodally, occurred in two mobilities, the more anodal of which is interpreted as an a/a, the less anodal, as a b/b homozygote pattern. While b/b homozygotes yielded a slightly broader band, distinctly more intense in its anodal part, the a/a band was narrower, equally intense as the anodal part of the b/b band, and accompanied by a faint broad band in the cathodal part of the b/b band position. This is apparently due to the AK locus, which produces a band moving slightly slower than the CK b/b band, that is revealed also by the CK staining method. Reduced a/a plus b/b bands with a very intense intermediate band are interpreted as a/b heterozygote patterns. An interesting finding is that in non-specific protein staining (Coomassie Blue) of skeletal muscle extracts on low pH tris-citrate starch gels the protein yielding the most highly concentrated bands could be identified as CK: the mobility is identical with that of the bands observed in specifically staining for CK; and, more importantly, the electrophoretic phenotypes observed for the polymorphic, locally varying CK locus were identical, for each individual examined, with the patterns seen on the non-specifically stained gels (including the 664 individuals of the samples used for this study: 26 a/a and 536 b/b homozygotes, 102 a/b heterozygotes). This suggests caution in collectively classifying "general proteins" observed with non-specific stainings as non-enzymatic.

Allele distribution among samples used. The a allele is frequent to predominant in the Italian nonhybrid from the peninsular samples (Lavello and Tarsia), and was found as the non-ridibunda allele in a hybrid from San Donà. The b allele is the only allele found in Rana ridibunda, the Yugoslavian and the Greek taxon and the Italian nonhybrid from Sicily; it is the common allele of Rana lessonae (exclusively found at Zagreb, vastly predominating at San Donà), and is the ridibunda allele in all hybridogenetic hybrids discussed.

General allele distribution. The a allele occurs in varying frequency in Rana lessonae from western and central Europe and northern Italy, and is widespread and common in the usually polymorphic Italian nonhybrid throughout its peninsular range (Uzzell and Berger 1975; Graf, Karch and Moreillon 1977; Uzzell and Hotz 1979; Tunner and Heppich 1982; Hotz and Uzzell unpublished). The b allele characterizes Rana ridibunda and Rana lessonae in central and eastern Europe (Uzzell and Berger 1975; Graf, Karch and Moreillon 1977; Tunner 1980; Tunner and Heppich 1982; unpublished data) and Rana ridibunda in Anatolia (Uzzell 1982 b), and occurs in northern African water frogs (unpublished). The b allele has also been reported, without locality data, as typical for Rana perezi (Tunner and Heppich 1982); both in southern France and in the Iberian peninsula, however, this species much more commonly has different alleles (Graf, Karch and Moreillon 1977; Hotz and Uzzell unpublished), although it does have the b allele in low frequency (unpublished).

Synonyms. a: F (Uzzell and Berger 1975), 97 (Graf, Karch and Moreillon 1977), 103 (Tunner and Heppich 1982);

b: S (Uzzell and Berger 1975), 85 (Graf, Karch and Moreillon 1977), 100 (Tunner 1980).

α -GDH

Electrophoretic patterns. Highest activity of this locus was detected in skeletal muscle and liver tissues, less in kidney, and none in heart muscle. Six different multibanded phenotypes were observed on low pH tris-citrate gels, interpreted as being due to four alleles (a, b, c, d) coding for the subunits of a dimeric enzyme. Intense bands plus 1 to 4 equidistant serially less intense more anodal satellite bands occurred in two anodal mobilities, the

more anodal representing a/a, the less anodal, b/b homozygotes. In addition, four heterozygote phenotypes were observed. a/b individuals yielded 4 to 7-banded patterns that included a weak band at the b position, strongest bands both at the a position and midway between the a and b positions, and anodal subbands like those seen in a/a homozygotes; the higher intensity of the aa compared to the bb homodimer band is apparently due to an anodal subband of the ab heterodimer band that overlaps with the aa homodimer band. b/c individuals showed a weak cathodally moving band, a stronger band in the b position, a most intense band midway between the cathodal and the b band, and weak anodal subbands like those seen in b/b homozygotes. An a/c individual revealed a weak band in the cathodal c position, a strongest band at the b position, a strong band at the a position, weak bands in positions of the ab heterodimer band and the subbands seen in a/a homozygotes, and a very faint band in the bc heterodimer position; this pattern can be explained by the ac heterodimer band moving to the b position midway between the a and c positions and yielding a subband in the ab heterodimer position, and by a barely visible subband of the cc homodimer in the bc heterodimer position. A b/d individual showed a weak cathodal band, nearer to the origin than the cc homodimer band, a weak band in the bb homodimer position, a most concentrated band midway between them, and very weak anodal subbands like those seen in b/b homozygotes. Using high pH tris-citrate gels, mobilities of the a and b allelic products were so similar that a/a and b/b homozygotes and a/b heterozygotes were difficult to distinguish. The c and b allelic products, however, both moving towards the anode, had much slower mobilities; and b/c, a/c and b/d heterozygotes revealed distinct, sharply 3-banded patterns with the middle heterodimer band being most intense. Only a faint anodal smear appeared instead of the subbands seen on low pH gels. That this enzyme clearly has a dimeric subunit composition is interesting, because a probable monomer structure has been proposed for α -GDH in the closely related eastern Palearctic water frogs (Nishioka, Ueda and Sumida 1981 a). A small mobility difference was repeatably observed on high pH tris-citrate gels (b/b individuals were examined) between skeletal muscle and liver tissue samples, the latter leading to slightly more anodal bands (see also Pep-2, SOD).

Allele distribution among samples used. The a allele is unique for Rana ridibunda, monomorphic in all samples, and is the ridibunda allele in all hybridogenetic hybrid lineages. The b allele is characteristic for Rana lessonae (monomorphic at Zagreb, vastly predominant at San Donà), the Italian non-

hybrid (monomorphic in the peninsular samples, vastly predominant at Catania), the Yugoslavian taxon (monomorphic) and the Greek taxon (predominant). The c allele occurs in low frequencies in the Greek taxon, and was found as the lessonae allele in one hybrid from San Donà. The d allele was only found in one Italian nonhybrid from Catania.

General allele distribution. Rana ridibunda is characterized throughout its European range by the a allele (Uzzell and Berger 1975; Tunner and Heppich 1982; unpublished data), which is also contained in the ridibunda genomes of Rana esculenta, of the Italian hybrid (Uzzell and Hotz 1979 and unpublished) and of Rana ridibunda × Rana perezi hybrid lineages (unpublished). The b allele is shared as the most common allele by Rana lessonae throughout its range (Uzzell and Berger 1975; Tunner 1980; unpublished data), by the Italian nonhybrid (Uzzell and Hotz 1979; Tunner and Heppich 1982; unpublished data), by the Yugoslavian and by the Greek taxon (Hotz and Uzzell 1982; Tunner and Heppich 1982); it occurs at low frequencies in some populations, mainly eastern, of Rana perezi (Hotz and Uzzell 1982, unpublished data; see also Tunner and Heppich 1982), is present in Anatolian Rana ridibunda (Uzzell, Günther and Berger 1977; Uzzell 1982 b; unpublished data), and occurs exceptionally in central European Rana ridibunda (Uzzell and Berger 1975), possibly due to introgression from Rana lessonae (Uzzell 1982 b). The c allele is the most common allele of Rana perezi (Tunner and Heppich 1982, Hotz and Uzzell 1982; unpublished data), has been reported for the Greek taxon from Korfu (Tunner and Heppich 1982), and occurs in northern African water frogs (unpublished); it has not been found so far in Rana lessonae except in the single case from San Donà reported here. The d allele has not yet been found in other water frogs.

Synonyms. a: F (Uzzell and Berger 1975), 100 (Tunner 1980);

b: S (Uzzell and Berger 1975), 94 (Tunner 1980), 96 (Tunner and Heppich 1982);

c: 93 (Tunner and Heppich 1982);

d: none.

GDH

Electrophoretic patterns. No repeatable, interpretable variation has been found at this locus, which is most active in liver and kidney tissues, less so

in skeletal and heart muscle. On high pH tris-citrate gels, most individuals yielded a distinct, sharp, anodally moving band, without subbands, in a single position, suggesting one allele (a) of a largely monomorphic locus. A few frogs showed less distinct, smeary patterns, more often extending to the less anodal part of the a position. Because this results in uncertainties, this locus is not included in the main body of this study. Faint LDH bands and, if liver extracts were used, IDH-1 bands often appeared on gels stained for GDH (both NAD and NADP were used for staining).

Allele distribution among samples used. The a allele seems to be shared by all taxa from each region.

General allele distribution. The a allele occurs also in Rana perezii and in northern African water frogs (unpublished data).

Synonyms. None.

GPI

Electrophoretic patterns. GPI yielded similarly high activity in all tissues examined; it was usually scored from skeletal muscle samples. Nine different phenotypes, all moving cathodally, were observed on low pH tris-citrate gels. All patterns are consistent with the interpretation that 6 different alleles (a, b, c, d, f, g) code for the subunits of a dimeric enzyme. Homozygote patterns were found in two different mobilities (a/a, d/d); they consisted of a dense band plus 1 or 2 equidistant, distinct, serially weaker less cathodal subbands, those of the a/a band usually being more highly concentrated than those of the d/d band. In addition, 7 different heterozygote patterns were observed. a/d individuals yielded reduced homodimer bands in each of the a/a and d/d homozygote positions plus a usually most intense, distinct intermediate band midway between them or slightly nearer to the more cathodal dd homodimer band, and at least one subband as seen in a/a homozygotes. Five other similar 3-banded patterns included only one of the aa or dd homodimer bands, the other homodimer band was in one of four other, different positions (a/c, a/f, a/g, b/d, d/f heterozygotes). a/f heterozygotes yielded a faint band approximately midway between the aa homodimer and the af heterodimer band, apparently representing a less cathodal subband of the heterodimer band. a/b heterozygotes, due to the small mobi-

lity difference between the a and b allelic products, showed a broad band, most intense in the middle and with the less cathodal end at the aa homodimer position, rather than a distinct 3-banded pattern. Both the c and the g allelic products have a mobility so similar to that of the d allelic product occurring in the same taxa that c/d or d/g heterozygotes probably would be difficult to distinguish from the respective homozygotes.

Allele distribution among samples used. The a allele characterizes Rana ridibunda: it is polymorphic in each sample (being less frequent than the d allele at Zagreb, Skopje and Igoumenitsa, equally or more frequent at Metković and Skadarsko Jezero), is the only ridibunda allele in all hybrid lineages from the Italian samples, and occurs in low frequencies in Rana lessonae from San Donà and the Italian nonhybrid from Tarsia and Catania (judged by some hybrids yielding only the a electromorph, interpreted as a/a homozygotes; see also Hotz and Uzzell 1983). At low frequencies, the b allele is present in Rana ridibunda from Skopje and Igoumenitsa, and the c allele, in Rana ridibunda from Skadarsko Jezero. The d allele is shared by all taxa discussed; it is the most common allele of Rana lessonae (predominant at all localities) and of the Italian nonhybrid (monomorphic at Lavello, predominating at Tarsia and Catania), is the only allele found in the Yugoslavian and in the Greek taxon, and occurs in each sample of Rana ridibunda (being the most frequent allele at Zagreb, Skopje and Igoumenitsa). The f allele is present at low frequencies in Rana ridibunda from Skadarsko Jezero and Skopje, in Rana lessonae from Zagreb (judged by its occurrence in hybrids; see Appendix 4) and San Donà, and in the Italian nonhybrid from Tarsia. The g allele was found as the lessonae allele in a hybrid from San Donà-1.

General allele distribution. The a allele is common in the usually polymorphic central and eastern European Rana ridibunda (Uzzell and Berger 1975), is widespread in Greek Rana ridibunda (Tunner and Heppich 1982), is the ridibunda allele in hybrid lineages throughout Italy (Uzzell and Hotz 1979 and unpublished) and in Rana ridibunda × Rana perezi hybrid lineages (Hotz and Uzzell 1983; unpublished data), occurs at low to moderate frequencies in northern Italian Rana lessonae (unpublished) and is widespread but has low frequencies in the Italian nonhybrid (unpublished data; see also Uzzell and Hotz 1979). The c allele is present at low frequencies in Rana ridibunda from other parts of Adriatic southwestern Yugoslavia (Hotz and Uzzell 1982). The d allele is characteristic for Rana lessonae throughout its range (Uzzell and Berger 1975, Tunner and Heppich 1982; unpublished data), for the Italian

nonhybrid (Uzzell and Hotz 1979), for the Yugoslavian and the Greek taxon throughout their ranges (Hotz and Uzzell 1982, Tunner and Heppich 1982, Uzzell and Hotz unpublished), and occurs in Rana ridibunda: in varied frequencies throughout its European range (Uzzell and Berger 1975; Graf and Müller 1979; Tunner and Heppich 1982; unpublished data), commonly in Anatolia (Uzzell, Günther and Berger 1977; Uzzell 1982 b; unpublished data) and in the easternmost parts of its range (Kazakhstan; Hotz and Uzzell unpublished). The d allele has also been reported as the common allele of Rana perezi (Tunner and Heppich 1982); the low pH tris-citrate gels reveal, however, that the most common allelic product found in Rana perezi throughout its range as well as in Rana saharica (with a mobility similar to that of the d electromorph) is encoded by a different allele, e (Hotz and Uzzell 1982, 1983, unpublished). The allele f is widespread, usually at low frequencies, in Rana ridibunda (Hotz and Uzzell 1982, Tunner and Heppich 1982; unpublished data), occurs at low frequencies in Rana lessonae in different parts of its range (Tunner 1980, Tunner and Heppich 1982; unpublished data) and is widespread though locally infrequent in the Italian nonhybrid (Hotz and Uzzell unpublished). The b and g alleles have not yet been found elsewhere in water frogs.

Synonyms. a: S (Uzzell and Berger 1975), -44 (Graf and Müller 1979), 96 (Tunner 1980), 93 (Tunner and Heppich 1982);

d: F (Uzzell and Berger 1975), -100 (Graf and Müller 1979), 100 (Tunner 1980);

f: 102 (Tunner 1980);

b, c, g: none.

IDH-1

Electrophoretic patterns. This, the more anodal of two IDH loci, yielded high activity in liver and kidney tissues, low activity in skeletal and heart muscle. Three different phenotypes, moving anodally, were observed on high pH tris-citrate gels, interpreted as produced by three alleles (a, b, c) coding for the subunits of a dimeric enzyme. Single distinct intense bands without subbands apparently represent a homozygote pattern (b/b). In addition, two different heterozygote patterns were seen: a/b individuals yielded broader, smeary bands, extending from the b position (with reduced concentration) towards the anode, most intense in the middle, without the aa

homodimer band being distinctly separated from the heterodimer band; b/c individuals showed a slightly smeary, 3-banded pattern with a reduced band both in the b position and in a less anodal position plus a more intense band midway between them. IDH-1 appeared as faint bands also on high pH tris-citrate gels stained for several other enzymes (GDH, 6PGDH, MPI, PGM, glucose-6-phosphate dehydrogenase, hexokinase, hydroxybutyrate dehydrogenase), if NADP was present in the staining solution or in the gel, and if liver samples were used; it was identified on these gel slices by its mobility and by the few b/c heterozygote individuals, which yielded the same pattern as in specific staining for IDH.

Allele distribution among samples used. All samples of Rana ridibunda, the Yugoslavian and the Greek taxon are monomorphic for the b allele, which is also the common allele of Rana lessonae and of the Italian nonhybrid (the only one found at Tarsia and Catania, the more frequent one at Lavello). The a allele was found as a/b heterozygotes in a Rana esculenta from Zagreb and in an Italian hybrid from Tarsia; in neither case can it be determined to which parental genome it belongs (Appendix 4). It is unknown whether this indistinguishable mobility found at such low frequencies in two distant areas reflects allelic identity or convergence; a direct comparison of the two a/b patterns is difficult, because the aa homodimer was not visualized as a distinct band. The c allele occurs in low frequencies in the Italian nonhybrid at Lavello and was found in a Rana esculenta from San Donà.

General allele distribution. The c allele seems to occur in other localities of Adriatic central Italy (unpublished); other allele distributions are not yet established.

Synonyms. None.

IDH-2

Electrophoretic patterns. This, the much less anodal of two independent IDH loci, was highly active in skeletal and heart muscle tissues, but showed very low activity in liver and kidney extracts. Two different phenotypes, moving anodally, were seen on high pH tris-citrate gels, apparently due to two alleles (a, b). All but one individual uniformly yielded a narrow, sharp, distinct intense band without subbands. This is interpreted as a homozygote pattern

(b/b) of a largely monomorphic locus. A single frog revealed broader, somewhat smeary bands, extending from the b/b homozygote position, with reduced intensity, towards the anode, most concentrated in the middle. This apparently represents a heterozygote pattern (a/b) of a non-monomeric, possibly dimeric enzyme.

Allele distribution among samples used. The b allele is shared by all individuals of each taxon. The a allele occurred in a single heterozygous Rana esculenta from San Donà-2; it cannot be conclusively attributed to either the lessonae or the ridibunda parental genome (Appendix 4).

General allele distribution. The common b allele is also shared by Rana perezi and by northern African water frogs (unpublished data).

Synonyms. None.

LDH-1

Electrophoretic patterns. This locus, corresponding to the B locus (Markert 1963, Wright and Moyer 1966), revealed an increasingly high activity of its products, relative to that of the LDH-2 (= A) locus products, in skeletal muscle, kidney, liver and heart muscle tissues. It was scored using the most anodal zone of LDH activity containing homotetramers of the B locus (Markert 1963), usually from skeletal muscle tissues. Ten different phenotypes were distinguished, moving anodally on low pH tris-citrate gels, interpreted as being due to 6 alleles (a, b, c, d, e, f). Within this B⁴ homotetramer zone, single sharp, intense bands occurred in 5 mobilities, representing homozygote patterns (a/a, b/b, c/c, d/d, e/e); distinct 5-banded patterns, the least intense fastest and slowest bands in two of these homozygote band positions, the central band most intense, represent 2 kinds of heterozygotes (a/c, a/d); and more smeary 5-banded patterns or indistinctly banded broad smears, the least intense anodal and cathodal ends in two other homozygote band positions, most concentrated in the center, represent 2 further kinds of heterozygotes (b/c, c/e). The d and e allelic products had very similar mobility on low pH tris-citrate gels (d slightly faster), but differed markedly in mobility both on Poulik gels and on polyacrylamide gels (d considerably slower). Finally, a very different 3-banded pattern was repeatably observed within the B⁴ homotetramer zone in several individuals: it consisted of 3

equidistant bands, the slowest in the $\underline{c}/\underline{c}$ homozygote band position, the fastest slightly farther from the origin than the $\underline{a}/\underline{a}$ homozygote band; the fastest band was least intense, the middle band as intense as or more intense than the slowest band. The same pattern was observed in these individuals using plasma LDH on polyacrylamide gels, except that the relative intensity of the 3 bands serially decreased towards the anode. This phenotype, only seen in Italian hybrids, is interpreted as a $\underline{c}/\underline{f}$ heterozygote pattern. That the least anodal band represents a $\underline{c}^4$ homotetramer is shown, other than by its identical mobility, by the exclusive expression of this ridibunda allele in gonads of the hybridogenetic hybrids (Appendix 4). It seems most plausible that the middle band represents a $\underline{c}^2\underline{f}^2$ heterotetramer and the most anodal band a $\underline{f}^4$ homotetramer, the asymmetrical heterotetramers not being expressed (possibly because of subunit assembly restrictions or lowered stability or enzymatic inactivity); this is consistent with the relative intensities observed (the most anodal band least concentrated). Analogous patterns have been reported for other vertebrates (for example Shaklee 1975). Since this Italian nonhybrid allele has only been found in hybrids, the position of the $\underline{f}^4$ homotetramer needs verification, and it cannot presently be excluded that the middle band represents a $\underline{c}^3\underline{f}^1$, the anodalmost band a $\underline{c}^2\underline{f}^2$ heterotetramer.

Allele distribution among samples used. The $\underline{a}$ and $\underline{c}$ alleles are unique to Rana ridibunda (strongly polymorphic at Zagreb, monomorphic for $\underline{a}$ at Metković, Skadarsko Jezero and Igoumenitsa, $\underline{a}$ vastly predominating at Skopje); the $\underline{c}$ allele is the ridibunda allele in each individual of all hybridogenetic hybrid lineages. The $\underline{b}$ and $\underline{e}$ alleles are characteristic for Rana lessonae (strongly polymorphic at Zagreb and San Donà), the $\underline{e}$ allele also for the Italian nonhybrid (predominating at Lavello and Tarsia, monomorphic at Catania). The $\underline{d}$ allele is shared by the Yugoslavian and the Greek taxon (both monomorphic). The $\underline{f}$ allele is present in low frequency in Italian nonhybrid genomes of hybrid lineages from the peninsular samples (Lavello, Tarsia).

General allele distribution. This locus has been extensively studied in western Palearctic water frogs (Uzzell and Berger 1975; Uzzell, Berger and Günther 1975; Günther and Hähnelt 1976; Vogel and Chen 1976, 1977; Nevo 1976; Uzzell, Günther and Berger 1977; Graf, Karch and Moreillon 1977; Vogel 1977; Uzzell and Hotz 1979; Tunner 1979, 1980; Kawamura and Nishicka 1979; Graf and Müller 1979; Uzzell, Hotz and Berger 1980; Hotz and Uzzell 1982, 1983; Uzzell 1982 b; Tunner and Heppich 1982; Binkert, Borner

and Chen 1982). The a allele is characteristic for Rana ridibunda, being usually the more common of two alleles segregating in autochthonous central and eastern European populations (Uzzell and Berger 1975, Günther and Hähnel 1976), predominating in southeastern Europe (Tunner and Heppich 1982), occurring in Anatolia (Uzzell 1982 b, Uzzell and Hotz unpublished) and in the easternmost parts of the species' range (Kazachstan; see Hotz and Uzzell 1982); it is one of two ridibunda alleles in central and eastern European Rana esculenta lineages (Uzzell and Berger 1975; Günther and Hähnel 1976; Vogel and Chen 1977; Graf, Karch and Moreillon 1977; Vogel 1977; Uzzell, Hotz and Berger 1980; Binkert, Borner and Chen 1982; Hotz and Uzzell 1983 and unpublished data); it is the ridibunda allele in Rana ridibunda × Rana perezi hybrid lineages in southern France (Graf, Karch and Moreillon 1977; Hotz and Uzzell 1983 and unpublished); and it has been reported for central European Rana lessonae in low frequency (Günther and Hähnel 1976), possibly introgressed from Rana ridibunda (Günther and Hähnel 1976; see also Uzzell, Günther and Berger 1977; Uzzell 1982 b). The second allele typical for Rana ridibunda is c, existing in this species at varying, usually minor frequency in autochthonous populations of central and eastern Europe (Uzzell and Berger 1975, Günther and Hähnel 1976), more rarely in the southern Balkans (Tunner and Heppich 1982; unpublished data), more commonly in Anatolia (Uzzell 1982 b; Uzzell and Hotz unpublished); it is the other ridibunda allele in central and eastern European Rana esculenta lineages (Uzzell and Berger 1975; Günther and Hähnel 1976; Vogel and Chen 1976; Graf, Karch and Moreillon 1977; Vogel 1977; Tunner 1979, 1980; Uzzell, Hotz and Berger 1980; Binkert, Borner and Chen 1982; Hotz and Uzzell 1983 and unpublished), and is the only ridibunda allele found in northern Italian Rana esculenta and in the Italian hybrid lineages (Uzzell and Hotz 1979, unpublished data). The c allele has also been reported to occur in low frequency in Rana perezi from southern France (Graf, Karch and Moreillon 1977); an allele with this mobility is widespread in this species in the Iberian peninsula, more common in the west (Hotz and Uzzell unpublished). The b and e alleles are typical for the usually polymorphic Rana lessonae throughout its range, b mostly in minor frequency (Uzzell and Berger 1975; Günther and Hähnel 1976; Vogel and Chen 1976, 1977; Graf, Karch and Moreillon 1977; Vogel 1977; Uzzell, Hotz and Berger 1980; Tunner 1980; Tunner and Heppich 1982; Binkert, Borner and Chen 1982; Hotz and Uzzell 1983; unpublished data); both b and e have been found at low frequency in central European Rana ridibunda (Uzzell and Berger 1975, Günther and Hähnel 1976), possibly introgressed from Rana lessonae (Günther and Hähnel

1976; Uzzell, Günther and Berger 1977; Uzzell 1982 b). The e allele also is characteristic for the Italian nonhybrid, being predominant or fixed throughout its range (Uzzell and Hotz 1979, Tunner and Heppich 1982; Uzzell and Hotz unpublished); the b allele occurs in low frequency in the Adriatic coastal area in the extreme northeast of the presumed Italian nonhybrid range (Fig. 1,3), possibly introgressed via migration from the Rana lessonae range (unpublished data). The d allele seems to be fixed both in the Yugoslavian and the Greek taxon (Hotz and Uzzell 1982, Tunner and Heppich 1982), it is the commonest allele of Rana perezi in most parts of its range (Graf, Karch and Moreillon 1977; Hotz and Uzzell 1982, 1983; Tunner and Heppich 1982; unpublished data) and is widespread in northern African water frogs (Uzzell and Hotz unpublished). The f allele almost certainly corresponds to the allele reported as F_1 (=b) for Tarsia by Uzzell and Hotz (1979; see Uzzell in press) and was found in the Italian nonhybrid genome of a hybrid in Adriatic central Italy (Uzzell and Hotz unpublished).

Synonyms. a: F_r , R_f (Uzzell and Berger 1975), LDB^c (Vogel and Chen 1976), LDB^d (Vogel and Chen 1977), 100 (Graf, Karch and Moreillon 1977), B^E (Kawamura and Nishioka 1979), 101 (Tunner 1980);

b: F_l , L_f (Uzzell and Berger 1975), LDB^c (Vogel and Chen 1976, 1977), 91 (Graf, Karch and Moreillon 1977), 100 (Tunner 1980);

c: S_r , R_s (Uzzell and Berger 1975), LDB^b (Vogel and Chen 1976), 86 (Graf, Karch and Moreillon 1977), B^C (Kawamura and Nishioka 1979), 97 (Tunner and Heppich 1982);

d: 71 (Graf, Karch and Moreillon 1977), 93 (Tunner and Heppich 1982);

e: I_l , L_i (Uzzell and Berger 1975), LDB^a (Vogel and Chen 1976), 75 (Graf, Karch and Moreillon 1977), B^B (Kawamura and Nishioka 1979), 95 (Tunner 1980);

f: F_l (Uzzell and Hotz 1979).

LDH-2

Electrophoretic patterns. This locus (= LDH-A: Markert 1963) showed increasingly high activity of its translation products in heart muscle, liver, kidney and skeletal muscle tissues. It was scored using the least anodal A^4 homotetramer zone, usually from liver samples on high pH tris-citrate gels. Two different phenotypes were observed, interpreted as being due to 2 alleles (a, b) coding for the subunits of this tetrameric enzyme (Markert 1963). A

single sharp intense band in most cases delimited the anodally moving zones of LDH activity towards the origin; this represents a homozygote pattern (a/a). Some individuals yielded instead a 5-banded pattern, with a much reduced fastest band in the a/a position, the other equidistant bands with slower mobility, the middle band being most concentrated; this represents an a/b heterozygote pattern. The least anodal band of b⁴ homotetramers was situated near the origin on high pH tris-citrate gels and moved cathodally on low pH gels.

Allele distribution among samples used. The a allele is shared by all taxa from each region. The b allele was only found in Rana ridibunda, occurring in low frequency at Zagreb and at Skopje.

General allele distribution. The a allele of this largely monomorphic locus is common to all western Palearctic water frog taxa, including Rana perezi and Rana saharica (see Uzzell and Berger 1975; Günther and Hähnel 1976; Vogel and Chen 1976; Graf, Karch and Moreillon 1977; Vogel 1977; Tunner 1980; unpublished data). According to Kawamura and Nishioka (1979) the same a allele seems to be shared also by the eastern Palearctic water frog group. The b allele has not yet been found elsewhere in water frogs.

Synonyms. a: 100 (Tunner 1980);

b: none.

MDH-1

Electrophoretic patterns. Comparably high activity of products of this locus was seen in skeletal and heart muscle, liver and kidney tissues. Five different multibanded phenotypes were observed, moving anodally on low pH tris-citrate gels, interpreted as being due to 3 alleles (a, b, c) coding for the subunits of a dimeric enzyme. b/b homozygotes yielded, on low pH gels refrigerated without being covered by pans of ice, a broad dense major band, distinctly more concentrated near its anodal end, plus 1 to 4 serially less intense equidistant anodal subbands and often one weak cathodal subband; on gels cooled by pans of ice, two separated, narrower bands were observed instead of the single broad band, the more anodal more concentrated; this most intense band apparently represents the main band of MDH-1 b/b homozygotes, whereas the less intense less anodal band, uniformly

occurring among all individuals examined, is due to the monomorphic MDH-2 locus. a/a homozygotes had a most intense band in a much more anodal position (a/a), showed no activity in the main b/b band position, had a very weak band about midway between the MDH-2 band and the main a/a band (presumably an anodal subband of the MDH-2 band) and 1 to 2 equidistant less intense anodal subbands of the main a/a band. a/b heterozygotes (*Rana ridibunda* × Greek taxon hybrids; see Hotz and Uzzell 1982) showed reduced bands both in the main a/a and b/b band positions, a more intense band midway between them (the ab heterodimer band), anodal subbands like those seen in a/a homozygotes, but weaker, and a faint subband on the cathodal side of the MDH-2 band. Tunner and Heppich (1982), using 12 % starch gels (pH 7) and polyacrylamide gels, reported a different banding pattern for a/a water frogs that seemed not to conform to known models of molecular subunit interaction: they observed 2 heavily stained MDH bands in b/b animals (one interpreted as MDH-1, the other, as MDH-2 products), and in a/a animals an additional band anodal but close to the MDH-1 band, exhibiting equal intensity. Tunner and Heppich proposed that this pattern might result from a gene duplication of an MDH locus, the products of which show no molecular interaction. The differences between their results and the data reported here, both sets of phenotypes consistently repeatable, clearly need further investigations; a comparison with the patterns observed in eastern Palearctic water frogs (Nishioka, Ohtani and Sumida 1980; Nishioka, Ueda and Sumida 1981 a,b) presumably will be helpful for interpretation. The clearly dimeric patterns seen in interspecific hybrids the parental species of which locally exhibit only a/a and b/b phenotypes, respectively, suggest, however, that the interpretation of two alleles of one of at least two MDH loci is plausible. Two other similar phenotypes were found in Italian water frogs: c/c homozygotes showed an intense MDH-2 band and an intense much less anodal band never seen in b/b animals (the main c/c band), revealed no activity in the main b/b band position, had 1 to 2 anodal subbands like b/b animals, but weaker, 1 to 2 equidistant serially weaker cathodal subbands of the c/c band, and a faint band between the main c/c and the MDH-2 band (presumably an anodal subband of the c/c band). b/d heterozygotes showed a combination of all bands seen in b/b and c/c homozygotes, although with reduced concentration, plus an intense band midway between the main b/b and c/c bands, apparently the bc heterodimer band.

Allele distribution among samples used. The a allele is unique to the Greek taxon (monomorphic). The b allele is the only allele found in *Rana ridibunda*

of each sample, is the usual allele in Rana lessonae (monomorphic at Zagreb, vastly predominant at San Donà), is the common Italian nonhybrid allele in peninsular Italy (monomorphic at Lavello, the more frequent allele at Tarsia), is the only allele found in the Yugoslavian taxon, and is the ridibunda allele in all hybridogenetic hybrid lineages. The c allele is the only allele found in the Italian nonhybrid from Sicily (Catania) and occurs in moderate frequencies at Tarsia; judged by electrophoretic mobility, the c allele also occurs in the lessonae genome of a hybrid from San Donà-1.

General allele distribution. The a allele is unique to and apparently fixed in the Greek taxon throughout its presently known range (Hotz and Uzzell 1982, Tunner and Heppich 1982). The b allele is the only allele found in central European Rana ridibunda and Rana lessonae (Tunner 1979, 1980, Tunner and Heppich 1982, Hotz and Uzzell 1982, unpublished data), occurs regionally in the Italian nonhybrid, often being the most common or only allele found (Tunner and Heppich 1982), especially in the northern and central peninsula, but seems not to occur in the Italian nonhybrid on Sicily (Uzzell and Hotz unpublished); it is the ridibunda allele in all hybridogenetic hybrid lineages (Hotz and Uzzell unpublished); and it also is common in Rana perezi (Tunner and Heppich 1982, Hotz and Uzzell 1982, unpublished data). The c allele is so far only known for the Italian nonhybrid, occurring on the peninsula in frequencies increasing towards the south and apparently fixed on Sicily (Uzzell and Hotz unpublished).

Synonyms. a: "pattern type II" (Tunner and Heppich 1982);

b: S (Tunner 1979), 100 (Tunner 1980), "pattern type I" (Tunner and Heppich 1982);

c: none.

MDH-2

Electrophoretic patterns. Products of this locus showed activity in skeletal and heart muscle, liver and kidney tissues; relative to the MDH-1 locus, MDH-2 bands were most intense in heart muscle, least intense in skeletal muscle samples. No variation was detected at this locus. A single intense band, moving anodally on low and on high pH tris-citrate gels was seen, mainly using skeletal muscle tissues, in all animals, independent of their MDH-1 phenotype. For this reason, this uniform band is here interpreted as

being produced by a single allele (a) of the monomorphic MDH-2 locus. It appeared either as a separate distinct band slightly less anodal than the MDH-1 main b band, or as the less intense cathodal part of a broad major band in MDH-1 b/b animals (see MDH-1). A faint anodal subband of the MDH-2 band was revealed in MDH-1 a/a and c/c homozygotes. Tunner (1980) reported an MDH-2 band only for adult water frogs, whereas the band described here appeared in individuals of all postlarval age classes; additional work is needed to determine whether Tunner and I are describing the same phenotype.

Allele distribution among samples used. The a allele is shared by all individuals of each taxon.

General allele distribution. The a allele is common to Rana ridibunda, Rana lessonae, the Italian nonhybrid, the Yugoslavian and the Greek taxon, Rana perezi and Rana saharica (unpublished data; see also Tunner and Heppich 1982, who possibly refer to the same phenotype).

Synonyms. None.

MPI

Electrophoretic patterns. MPI activity was highest using skeletal muscle tissues, slightly lower in kidney, heart muscle and liver samples. A minimum of 19 different phenotypes were distinguished, all moving anodally on low pH tris-citrate gels, interpreted as representing at least 9 alleles (a, b, c, d, e, f, g, h, i) coding for a monomeric enzyme. Intense single bands with a less intense anodal subband (occasionally 2 equidistant subbands) occurred in 6 different mobilities, representing homozygote patterns (a/a, b/b, c/c, d/d, h/h, i/i). Two reduced bands in 2 of 9 different positions (including the 6 positions found as homozygotes), each with a weaker anodal subband, represent heterozygotes (11 kinds were distinguished: a/b, a/c, a/d, a/h, b/d, c/f, c/g, c/h, c/i, d/e, f/i). The more anodal of the two main bands of heterozygotes often was the more intense; this was especially evident in a/c and b/d heterozygotes, in which the subband of the slower band (c, d) had approximately the same mobility as the faster main band (a, b), this superposition resulting in a single highly concentrated faster band. b/b and i/i homozygotes were also distinguished from a/b and c/i heterozygotes

because their subband moved more anodally than the main a and c band, respectively; d/d and h/h homozygotes showed a subband moving less anodally than the main a band seen in a/d and a/h heterozygotes. The mobility difference between the h and i allelic products (both are Rana lessonae and Italian nonhybrid alleles that co-occur in the same samples) are so small that h/i heterozygotes are not clearly distinguishable from the respective homozygotes, although c/h and c/i heterozygotes are easily distinguished in hybrids. Finally, a different pattern was observed in the Sicilian samples: all hybrids showed a strongest band in the c position, slightly broader than usual at its cathodal end, an intense band in the f position and the respective anodal subbands; all nonhybrids had a strongest band in the f position and its subband, plus a less intense distinct band in the cathodalmost part of the broader c band seen in hybrids. Since each of 89 hybrids and 23 nonhybrids examined exhibited these patterns, it is difficult to explain the nonhybrid phenotype as a heterozygote pattern involving the f allele and a further allelic product (with slightly slower mobility than that of the c allelic product) in a population with Mendelian segregation. Although no conclusive interpretation is at present possible, it seems plausible to speculate that in these Sicilian nonhybrids the MPI locus may have undergone a gene duplication leading to fixed heterozygosity, and that hybrids therefore get one copy of each of two allelic duplicates from the nonhybrid parent plus the clearly present c allele in their ridibunda genome, clonally inherited from their hybrid parent. Because of these uncertainties, allelic frequencies for MPI are not determined for some Italian samples.

Allele distribution among samples used. The a allele is the most common Rana ridibunda allele (monomorphic at Metković, predominant at Skadarsko Jezero and Skopje, moderately frequent at Zagreb and Igoumenitsa). The b allele is shared by Rana ridibunda (in low frequency at Skadarsko Jezero and Skopje, more polymorphic at Igoumenitsa) and the Greek taxon (the most common allele). The c allele has only been found in Rana ridibunda (in low frequency at Skadarsko Jezero and Skopje, more common at Zagreb) and as the ridibunda allele in all hybridogenetic hybrid lineages of Italy. The d allele is predominant in the Yugoslavian taxon and occurs in lower frequencies in the Greek taxon. The e allele occurs at minor frequencies in the Yugoslavian taxon. The f allele was only found in Italian nonhybrid genomes (monomorphic at Catania - see above for a possibly fixed heterozygosity - and in moderate frequency at Tarsia). The g allele was only found in Italian nonhybrid genomes at Tarsia. The h allele characterizes Rana lessonae

(monomorphic at Zagreb, predominating at San Donà) and occurs in lower frequency in the Italian nonhybrid (Lavello and Tarsia). The i allele is the most common Italian nonhybrid allele in the peninsular samples (Lavello and Tarsia) and occurs at low frequency in Rana lessonae at San Donà.

General allele distribution. The a allele is widespread in central European and in other Balkan Rana ridibunda (Hotz and Uzzell 1982, unpublished) and is the ridibunda allele in Rana ridibunda × Rana perezi hybrid lineages of southern France (Hotz and Uzzell 1982). The distribution of the other alleles is not yet established.

Synonyms. None.

Pep-2

Electrophoretic patterns. Highest activity for this enzyme, which cleaves both leucyl-tyrosine and leucyl-valine, was revealed in liver and kidney tissues, less in skeletal, least in heart muscle. Seven different phenotypes, moving anodally on high pH tris-citrate gels, are interpreted as being produced by 4 alleles (a, b, c, d) coding for the subunits of a dimeric enzyme. Single intense broad bands without subbands occurred in 3 mobilities, apparently representing homozygote patterns (a/a, b/b, c/c). Heterozygote patterns, depending on the mobility difference between their two homodimer bands, either appeared as broad, slightly smeary bands beginning with reduced intensity at one of the homozygote positions and ending with equally reduced intensity at another of the homozygote or at a different position, with a discrete most intensely colored zone in the middle (a/b and c/d heterozygotes), or showed a distinct 3-banded pattern, the intermediate band most concentrated (b/c and especially a/d heterozygotes). Skeletal muscle samples repeatably revealed slightly less anodal bands than liver samples on the same gels (see also α -GDH, SOD).

Allele distribution among samples used. The a allele is present at low frequency in Rana ridibunda (Zagreb, Skopje); an allelic product with similar mobility was seen in a heterozygous specimen of the Greek taxon (Igoumenitsa). The b allele is the most common allele of Rana ridibunda in all samples (apparently fixed at Metković, Skadarsko Jezero and Igoumenitsa, predominant at Zagreb and Skopje), is the ridibunda allele in the hybrid

lineages of all samples, and apparently occurs at low frequency in Rana lessonae genomes (Zagreb, San Donà; judged by two Rana esculenta evidencing only the b band, interpreted as b/b homozygotes); the same allele apparently also characterizes the Greek taxon. The c allele is typical for Rana lessonae (vastly predominating at Zagreb and San Donà), for the Italian non-hybrid (apparently fixed in the peninsular samples, predominant at Catania) and for the Yugoslavian taxon (apparently fixed). The d allele is present at low frequency in the Italian nonhybrid from Sicily (Catania).

General allele distribution. The b allele is the most common allele in central European Rana ridibunda and is the ridibunda allele in central European Rana esculenta lineages; it apparently is also the more common allele of Rana perezi and occurs in northern African water frogs (unpublished data; see Hotz and Uzzell 1982). The c allele also characterizes central European Rana lessonae (Hotz and Uzzell 1982).

Synonyms. None.

PGM-2

Electrophoretic patterns. This less anodal of at least two PGM loci accounts for very strong bands in skeletal muscle and liver tissues, weaker bands in heart muscle, least intense bands in kidney samples. Ten different anodally moving phenotypes were observed on low pH tris-citrate gels; all patterns are consistent with the interpretation that 6 alleles (b, c, d, e, f, g) code for a monomeric enzyme. Intense distinct bands, usually with one weak anodal subband, occurred in 3 different mobilities, representing homozygote patterns (b/b, c/c, d/d). Two- to 4-banded patterns with a reduced band in each of two of the b, c and d positions, or in one of them and a different position, usually with weak anodal subbands, represent heterozygote patterns (b/c, b/d, b/e, b/f, c/d, d/f, d/g were found). In heterozygous hybrids, the band corresponding to the ridibunda allele (b or d) tended to be more concentrated than that due to the non-ridibunda allele (c, e or f). In b/d heterozygotes, the more anodal b band overlaps with the subband of the slower d band and is more concentrated.

Allele distribution among samples used. The b allele is the less common of two alleles characteristic for the largely polymorphic Rana ridibunda in each

region; it is the ridibunda allele in all hybridogenetic hybrid lineages of mainland Italy (San Donà, Lavello, Tarsia), and it is the only allele found in the Yugoslavian taxon samples. The c allele is typical for Rana lessonae (predominant at Zagreb and San Donà) and the Italian nonhybrid (apparently fixed at Tarsia, predominant at Lavello and Catania). The d allele is the most common allele of Rana ridibunda across all samples, is the ridibunda allele of Sicilian hybrid lineages (Catania), and was found at low frequency in lessonae genomes at Zagreb (judged by a Rana esculenta exhibiting only the d electromorph, interpreted as a d/d homozygote). The e allele is a rare Rana lessonae allele at San Donà. The f allele occurs at low frequency in the Italian nonhybrid genomes at Lavello and Catania. The g allele was found in one Rana ridibunda from Metković-1.

General allele distribution. The b and d alleles characterize central and eastern European Rana ridibunda, and occur as ridibunda alleles in central European Rana esculenta lineages, d being usually more common than b (Uzzell and Berger 1975; Tunner and Dobrowsky 1976; Uzzell, Hotz and Berger 1980); both alleles occur in Greek Rana ridibunda, d always in higher frequency (Tunner and Heppich 1982), and have been found, in equal frequency, in Anatolian Rana ridibunda (Uzzell 1982 b). The ridibunda allele in hybrid lineages throughout peninsular Italy is b (Uzzell and Hotz unpublished), while d is the ridibunda allele of Rana ridibunda × Rana perezi hybrid lineages in southern France (unpublished data). The b allele has been reported as the common Rana lessonae and as one of two common Italian nonhybrid alleles by Tunner and Heppich (1982), but this is apparently due to the use of a buffer system not resolving the mobility difference between the b and c allelic products (see also Tunner and Dobrowsky 1976, Tunner 1979, 1980), which is very marked on the low pH tris-citrate gels used for this study. The d allele has been reported as one of two common Italian nonhybrid alleles by Tunner and Heppich (1982), which is, however, inconsistent with data available for more than 600 water frogs throughout the Italian region in which this allele has never been observed (Uzzell and Hotz unpublished). The d allele was also reported as the common allele of Rana perezi (Tunner and Heppich 1982); although an enzyme with similar mobility as that of the d allelic product is common in Rana perezi and occurs in northern African water frogs, this might represent a different allele (unpublished data). The c allele characterizes Rana lessonae throughout its range (Uzzell and Berger 1975; unpublished data) and is also the most common Italian nonhybrid allele throughout its range (Uzzell and Hotz unpublished). The distribution of the other alleles is not yet established.

Synonyms. b: F (Uzzell and Berger 1975), probably S (Tunner and Dobrowsky 1976), probably I (Tunner 1979), probably 100 (Tunner 1980), 100 (Tunner and Heppich 1982);

c: I (Uzzell and Berger 1975), S (Tunner and Dobrowsky 1976), 100 (Tunner 1980, Tunner and Heppich 1982);

d: S (Uzzell and Berger 1975), L (Tunner and Dobrowsky 1976), 97 (Tunner 1980);

e,f,g: none (to be compared with 94: Tunner and Heppich 1982).

6PGDH

Electrophoretic patterns. Highest activity of this enzyme was found in liver and kidney tissues, slightly lower activity in skeletal and heart muscle samples. A minimum of 16 different phenotypes were observed, moving anodally on high pH tris-citrate gels, interpreted as being due to at least 5 different alleles coding for the subunits of a dimeric enzyme. Single intense sharp bands without subbands were seen in 4 mobilities, representing homozygote patterns (b/b, c/c, d/d, e/e). Two different types of heterozygote phenotypes were observed: distinct, clear, 3-banded patterns with two reduced outer bands, one or both of them in a position seen as homozygote patterns, and a more intense intermediate band (a/c, a/d, b/d, b/e, c/d, c/e, d/e heterozygotes were found); and very broad bands, with a reduced but distinct band in the d or (more reduced) in the e position, extending anodally in a indistinct smear, more intense in the center, approximately to the a or to the b band zone ("a"/d, "b"/d, "b"/e; all seen in hybridogenetic hybrids). A different smeary pattern was observed in Rana lessonae and some Italian nonhybrids: a broad band approximately between the b and c positions, more intense in the middle. All these unclear phenotypes were repeatably exhibited, in different tissues, by the same individuals. Finally, all individuals of the Yugoslavian and of the Greek taxon repeatably showed broad, smeary bands extending from the d position, with reduced intensity, towards the anode, most concentrated in the center. Because of these uncertainties, no allelic frequencies are determined for most samples of Rana lessonae and Italian nonhybrid genomes and for the Yugoslavian and the Greek taxon. Although no conclusive explanation can presently be offered for the smeary patterns observed, it seems plausible that again, as possibly for MPI, gene duplications in genomes of Rana lessonae, the Italian nonhybrid, the Yugoslavian and the Greek taxon are involved.

Allele distribution among samples used. The a allele occurs at low frequency in Italian nonhybrids from Catania, Tarsia (partly as "a") and possibly in Rana lessonae ("a"). The b allele is the most common allele in Italian non-hybrid genomes at Tarsia (sometimes "b") and Lavello (mostly "b"), and in Rana lessonae genomes ("b"). The c allele occurs at low frequency in Rana ridibunda (Zagreb, Skopje, Igoumenitsa); judged by electrophoretic mobility, the same allele occurs in the Italian nonhybrid (predominant at Catania, in medium frequencies at Tarsia, less frequent at Lavello). The d allele is characteristic for Rana ridibunda (medium frequency at Zagreb, predominant in all other regions), is the ridibunda allele in hybridogenetic hybrid lineages at Tarsia and Catania, and is one of two ridibunda alleles in hybridogenetic lineages at Zagreb, San Donà (less frequent) and Lavello (more frequent). The e allele is also typical for Rana ridibunda (medium frequency at Zagreb, low frequency at Skopje), and is the second ridibunda allele present in Rana esculenta (Zagreb, San Donà) and Italian hybrid lineages (Lavello).

General allele distribution. Not yet established.

Synonyms. None.

SOD

Electrophoretic patterns. This enzyme was scored on all gels stained using MTT-PMS linked reactions; higher activity was observed in liver and kidney tissues than in skeletal and heart muscle samples. Two different phenotypes, with fast anodal mobility both on low and high pH tris-citrate gels, were distinguished, interpreted as being produced by 2 alleles (a, b) coding for the subunits of a dimeric enzyme. All individuals but one showed an intensely decolored band on dark background plus a less intense anodal sub-band; this represents a homozygote pattern (a/a) of a largely monomorphic locus. A single individual exhibited a very reduced band in the main a/a band position, a least intense band with much slower mobility, and a most intense band approximately midway between the two reduced bands; this apparently represents a heterozygote pattern (a/b). Skeletal muscle samples repeatably revealed slightly less anodal bands than liver samples on the same gels (see also α -GDH, Pep-2), and primary oocytes yielded faster moving bands, on the same gels, than somatic tissues.

Allele distribution among samples used. The a allele is shared by all taxa from each region. The b allele was found in a Rana ridibunda from Skadarsko Jezero-1.

General allele distribution. The a allele is shared by all European water frog taxa (Uzzell, Hotz and Berger 1980; unpublished data) and occurs as one of two common alleles in northern African water frogs (Hotz and Uzzell unpublished). The b allele has not yet been reported for water frogs.

Synonyms. None.

MProt1

Electrophoretic patterns. This locus was scored using skeletal muscle extracts on polyacrylamide gels, in a zone slightly less anodal than the albumin zone. At least 3 1- or 2-banded phenotypes were observed, interpreted as being due to at least 2 alleles (b, c) coding for a monomeric protein. Very intense bands occurred in 2 mobilities, apparently representing homozygote patterns (b/b, c/c). Two equally dense reduced bands both in the b and c position, sometimes merging (with reduced concentration) in the middle, represent b/c heterozygotes. The c/c band usually appeared together with a very weak (adults) to much more distinct (immatures) narrower band slightly anodal of the b/b position; this apparently represents an age-specific expression of an independent locus. This pattern, observed in an immature c/c Greek taxon frog, was previously interpreted to represent an a/c heterozygote (Hotz and Uzzell 1982); the present interpretation is, however, conclusively supported by the immature b/c heterozygotes examined that exhibited, in addition to the two reduced b and c bands, the same distinct anodal band. A b/b band slightly broader on its anodal side than usual was seen in some partly adult Rana esculenta from Zagreb and may indicate the presence of yet another allele.

Allele distribution among samples used. The b allele characterizes Rana ridibunda, Rana lessonae and the Yugoslavian taxon in all samples; it also is the ridibunda allele in all Italian hybrid lineages, and it occurs at low frequency in the Italian nonhybrid at Lavello. The c allele is shared by the Greek taxon (apparently fixed) and the Italian nonhybrid (apparently fixed at Tarsia and Catania, predominant at Lavello).

General allele distribution. The b allele characterizes central European Rana ridibunda and Rana lessonae and occurs, in minor frequencies, in the Italian nonhybrid (unpublished data); it is the ridibunda allele in Rana ridibunda × Rana perezi hybrid lineages in southern France (unpublished). The c allele of the Italian nonhybrid and the Greek taxon is not distinguished by electrophoresis from a Rana perezi allele (see Hotz and Uzzell 1982).

Synonyms. c: a (Hotz and Uzzell 1982);

b: none.

MProt2

Electrophoretic patterns. No variation was detected at this locus, scored from skeletal muscle extracts on polyacrylamide gels. A distinct, uniform, concentrated band, similar in width and intensity to homozygous MProt1 patterns, was observed in a position about one third less anodal than the MProt1 zone. This is interpreted as representing a single allele (a) of a monomorphic locus. Immature, mainly newly metamorphosed frogs often exhibited 1-2 weak thin bands in constant positions between MProt1 and MProt2, apparently due to age-specific, possibly larval, expression of independent loci.

Allele distribution among samples used. The a allele is shared by all individuals of each taxon.

General allele distribution. The a allele also occurs in Rana perezi (unpublished data).

Synonyms. None.

MProt3

Electrophoretic patterns. No variation was observed at this locus, scored from skeletal muscle extracts on polyacrylamide gels. A distinct, sharp, narrow, intense band appeared in a anodal mobility of about 30 % relative to MProt1; it is interpreted as being due to a single allele (a) of a monomorphic locus. Usually this uniform band was accompanied by a less anodal, slightly

weaker and sometimes less clear band, apparently due to an independent locus.

Allele distribution among samples used. The a allele is shared by all individuals of each taxon.

General allele distribution. The a allele also occurs in Rana perezi (unpublished data).

Synonyms. None.

Alb

Electrophoretic patterns. Using plasma samples on polyacrylamide gels, at least 19 different anodal phenotypes were distinguished for this locus; all patterns are consistent with there being at least 9 alleles (a, b, c, d, e, f, g, h, i) coding for a monomeric protein. Intense sharp single bands occurred in 5 different mobilities, apparently representing homozygote patterns (a/a, b/b, c/c, e/e, g/g). In addition, at least 14 different double-banded heterozygote patterns were observed, either with reduced bands at each of two positions of these homozygotes (a/b, a/c, a/g, b/c, b/e), or at one of them plus at one of at least 4 different positions (a/f, a/h, a/i, c/d, c/possibly i, g/h, g/i), or at each of two of these 4 positions not found as single bands (f/h, f/i). In these heterozygote patterns, the more anodal of the two bands usually was the more intense, both for intraspecific heterozygotes (a/f, a/g, b/c, c/d, c/possibly i) and for interspecific hybrids (a/b, a/c, a/h, a/i, b/e, f/h, f/i, g/h, g/i). Some individuals of Rana ridibunda and the Yugoslavian taxon repeatably showed more diffuse patterns around the b/b position; these may in part represent b/h heterozygotes (the b and h allelic products are so similar in mobility that no distinct double-banded pattern is to be expected for heterozygotes), and in part yet a further allele may be involved, the product of which also moves only slightly differently from that of the b allele. Because of this uncertainty, no allelic frequencies are determined for Rana ridibunda and the Yugoslavian taxon. Two Rana esculenta from San Donà repeatably revealed, respectively, very slightly more closely and very slightly more widely spaced a and h bands than the other a/h heterozygotes; and a few Italian hybrids sometimes appeared to have a slightly more anodal mobility of their ridibunda albumin than that of

the i allelic product. Other methods are needed to investigate whether these differences are genetically determined.

Albumin was also scored using aqueous skeletal muscle extracts, obtained by sonication, on polyacrylamide gels. The phenotypes observed corresponded to those revealed by plasma samples (including the higher concentration of the more anodal band in heterozygotes), except that in most cases each band, whether in a homozygote or in a heterozygote pattern, appeared as a very closely spaced double band instead of as a single band. No explanation for this observation can presently be offered.

Allele distribution among samples used. The a allele is the most common allele of Rana lessonae (apparently fixed at Zagreb, vastly predominant at San Donà), and is also shared by the Italian nonhybrid (the most common allele at Lavello, one of two common alleles at Tarsia and at Catania). The b allele is one of the two most common Rana ridibunda alleles (probably fixed at Skadarsko Jezero and Igoumenitsa, in medium frequencies at Zagreb, Metković and Skopje); it also is the most common allele of the Yugoslavian taxon. The c allele is the second allele common in Rana ridibunda (the most frequent at Metković, highly polymorphic at Zagreb and Skopje). Allele d occurs at moderate frequency in Rana ridibunda at Skopje. The e allele is unique to the Greek taxon (apparently monomorphic). Allele f occurs in the Italian nonhybrid (one of two common alleles at Catania, in low frequency at Tarsia and Lavello) and at low frequency in Rana lessonae from San Donà. The g allele also occurs in the Italian nonhybrid from the peninsular samples (in moderate frequency at Lavello, highly polymorphic at Tarsia), and at low frequency in Rana lessonae at San Donà. The h allele was found as the ridibunda allele in two Rana esculenta from Zagreb-2 and probably occurs in Rana ridibunda at Zagreb in modest frequency and perhaps in other regions (see above for scoring difficulty); it is the ridibunda allele in Rana esculenta lineages at San Donà. The i allele characterizes the ridibunda genomes in the Italian hybrid lineages; an albumin with mobility not clearly distinguishable exists at minor frequency in Rana ridibunda at Metković and may represent the same allele.

General allele distribution. This is the most extensively studied locus among western Palearctic water frogs, both by electrophoresis (Tunner 1970, 1972, 1973 a, b, 1974, 1979, 1980; Engelmann 1972, 1973; Tunner and Uzzell 1974; Tunner and Dobrowsky 1976; Nevo 1976; Nevo and Yang 1979; Ebendal 1976, 1977; Wijnands and Gelder 1976; Wijnands 1977, 1978; Hemmer 1977;

Tunner in Uzzell and Hotz 1979; Günther and Lübcke 1979; Kyriakopoulou-Sklavounou 1980; Hemmer, Konrad and Bachmann 1980; Hotz and Uzzell 1982; Ebendal and Uzzell 1982; Uzzell and Tunner in press) and by immunology (Uzzell 1979 a, b, 1982 a, b, in press; Hotz and Uzzell 1982; Ebendal and Uzzell 1982; Uzzell and Tunner in press); data summarized here refer to these papers. The a electrophoretic allele characterizes Rana lessonae throughout its range and is the most common allele of the Italian non-hybrid; by immunology, however, the common allele of Rana lessonae is distinct from that of the Italian nonhybrid (Uzzell 1979 b, 1982 a,b, in press, and personal communication). The a allele has also been reported to occur at low frequency in central European Rana ridibunda, possibly due to introgression from Rana lessonae (Günther and Lübcke 1979). The b (possibly and/or h) and c alleles are the most common alleles distinguished in European Rana ridibunda, and have also been reported for the easternmost parts of the species' range (Kazakhstan; Günther and Lübcke 1979); both of them have been reported to occur at low frequency in central European Rana lessonae, possibly due to introgression from Rana ridibunda (Tunner and Dobrowsky 1976, Günther and Lübcke 1979; it is, however, possible that Rana lessonae does have at low frequency a further allele, similar to the c allele in mobility of its product: unpublished data). The b allele, judged both by electrophoresis and by immunology (Hotz and Uzzell 1982), is also the most common allele of the Yugoslavian taxon. The d allele occurs in minor frequency in other Balkan Rana ridibunda (Kyriakopoulou-Sklavounou 1980; unpublished data). The e allele characterizes the Greek taxon; by immunology, it seems to be derived from the b allele (Hotz and Uzzell 1982). The f allele is widespread in the Italian nonhybrid, less frequent in the north (unpublished data); whether the allele reported by Günther and Lübcke (1979) to occur at moderate frequency in central European Rana lessonae, its product moving more anodally than that of the a allele, corresponds to the f allele is not yet established. The g allele is widespread in the Italian nonhybrid in peninsular Italy; its product has a mobility very similar to that of the Rana ridibunda b albumin, but has been shown by immunology to represent a different allele (Uzzell in press). The h allele of Rana ridibunda has not yet been reported elsewhere for water frogs; its product has a mobility similar to but less anodal than that of the b albumin variant, so that the b alleles reported for central and northern Europe need direct comparisons. The i allele characterizes ridibunda genomes of Italian hybrid lineages.

Probable synonyms. a: A₄ (Tunner 1970), A₁ (Tunner 1973 b), A (Wijnands and Gelder 1976), Les (Tunner and Dobrowsky 1976), L (Uzzell and Hotz 1979), L₆ (Günther and Lübcke 1979), 100 (Tunner 1980);

b (and/or h?): A₂ (Tunner 1970), Ar₁ (Tunner 1973 b), B (Wijnands and Gelder 1976, Kyriakopoulou-Sklavounou 1980), R₅ (Tunner and Dobrowsky 1976), Fr (Tunner 1979), R_f (Günther and Lübcke 1979), 98 (Tunner 1980);

c: A (Tunner 1970), Ar₂ (Tunner 1973 b), C (Wijnands and Gelder 1976, Kyriakopoulou-Sklavounou 1980), R_L (Tunner and Dobrowsky 1976), Sr (Tunner 1979), R₈ (Günther and Lübcke 1979), 97 (Tunner 1980);

d: D (Kyriakopoulou-Sklavounou 1980);

g: R_F (Uzzell and Hotz 1979);

i: R₃ (Uzzell and Hotz 1979);

i, f, h: none (f to be compared with L_f: Günther and Lübcke 1979; h to be compared with synonyms reported for b).

APPENDIX 3

PLOIDY: EVIDENCE FROM ERYTHROCYTE SIZE AND ELECTROPHORESIS

The size of erythrocytes can be used to determine ploidy in amphibians (Fankhauser 1945, Uzzell 1964; see also George and Lennartz 1980). This means has been successfully applied to central and northern European water frogs for discriminating diploid and triploid Rana esculenta (for example Uzzell and Berger 1975; Uzzell, Berger and Günther 1975; Günther 1977; Berger, Roguski and Uzzell 1978; Berger and Roguski 1978; Günther, Uzzell and Berger 1979; Berger and Truszkowski 1980). To determine whether triploids are contained in the samples discussed here, erythrocyte areas were estimated for 10 measured cells of each individual.

The results are summarized in Table 15. Slight differences were found in local populations of some taxa between age classes and between sexes. Adults of Rana ridibunda, the Italian taxon and the Greek taxon, for both sexes, usually had somewhat larger mean erythrocyte areas than immature individuals; the difference is significant for females of the Italian taxon from Lavello-1 ($p < .05$, Mann-Whitney U test) and from Catania-1 ($p < .01$). Erythrocytes of adults were also significantly larger than those of immatures in Rana esculenta from San Donà-2 ($p < .05$) and in the Italian hybrid of both sexes from Catania-1 ($p < .01$). Males of the Italian nonhybrid, whether immature or adult, had slightly larger erythrocytes than females; the difference was significant for immature individuals from Lavello-1 ($p < .05$). Erythrocytes of males were also slightly but insignificantly larger in samples of the Yugoslavian and Greek taxa.

There was considerable difference between regions within a taxon (Table 16) as well as between taxa (Tables 17 and 18). Erythrocyte areas decreased from north to south (Fig. 1) in Rana ridibunda, averaging largest in Zagreb and smallest in Igoumenitsa. This trend is also seen in the Italian hybrid and in comparing the Yugoslavian taxon with the Greek taxon (Tables 16, 18), and is concordant with the values reported for erythrocyte areas in central European water frogs, which average generally higher, for diploid adults of both sexes of each taxon, than any of the values reported here (Uzzell and Berger 1975, Günther 1977; and unpublished data). For each

Table 15. Summary of variation in erythrocyte size.

Mean erythrocyte areas (in μm^2 , calculated from 10 erythrocytes per individual) of immature and adult females and males of the replicate samples discussed. Upper line: number of individuals and observed range of means; lower line: mean of means and standard deviation of means. Sex was not determined for 6 immature hybrids (3 from San Donà-1, 1 from Lavello-2, 2 from Catania-1); their mean erythrocyte areas are 244, 225, 227, 251, 197, 247 μm^2 , respectively.

Taxon Locality	Females		Males		All individuals
	immature	adult	immature	adult	
<i>Rana ridibunda</i>					
Zagreb-1	6 228-268 250 14	4 220-250 241 14	5 249-270 257 10	6 229-272 255 19	21 220-272 251 15
Zagreb-2	4 255-276 265 10	1 270	8 235-299 267 20	8 250-333 287 32	21 235-333 274 25
Metković-1	9 216-294 256 29	3 244-268 258 12	6 230-276 253 16	8 214-300 259 33	26 214-300 256 25
Metković-2	7 228-272 243 15	10 232-289 246 17	6 223-276 246 20	3 227-269 251 22	26 223-289 246 17
Skadarsko Jezero-1	2 213-220 217 5	2 244-246 245 1	1 234	1 235	6 213-246 232 13
Skadarsko Jezero-2	3 235-241 239 4	1 240	13 205-258 237 15	3 240-275 260 18	20 205-275 241 16
Skopje-1	3 221-230 226 5	6 212-268 240 20	2 210-235 223 18	14 203-264 234 20	25 203-268 234 18
Skopje-2	3 215-264 237 25	16 217-271 247 15	4 238-255 248 7	18 219-281 246 18	41 215-281 246 16
Igoumenitsa-1	2 227-251 239 17	1 245	1 227	1 241	5 227-251 238 11
Igoumenitsa-2	4 182-254 213 30	4 192-238 220 20	3 196-224 212 14	7 212-236 225 9	18 182-254 219 18
<i>Rana lessonae</i>					
Zagreb-2	0	0	0	1 283	1 283
San Donà-1	0	1 231	0	0	1 231
San Donà-2	0	1 253	0	0	1 253

Table 15 (continued).

Taxon Locality	Females		Males		All individuals	
	immature	adult	immature	adult		
Italian nonhybrid						
Lavello-1	7 186-217	9 181-226	11 179-250	7 193-246	34 179-250	
	197 12	213 14	217 21	223 22	213 19	
Lavello-2	0	3 221-234	0	0	3 221-234	
		225 8			225 8	
Tarsia-1	0	1 209	0	1 239	2 209-239	
					224 21	
Catania-1	7 201-220	8 210-261	5 196-238	3 228-243	23 196-261	
	208 6	231 17	220 17	234 8	222 16	
Yugoslavian taxon						
Skadarsko Jezero-1	4 243-263	10 205-284	10 222-311	7 222-281	31 205-311	
	250 9	248 30	258 26	246 20	251 24	
Skadarsko Jezero-2	3 213-258	1 228	2 246-259	3 247-259	9 213-259	
	236 23		253 9	252 6	244 16	
Greek taxon						
Igoumenitsa-1	1 202	17 200-254	4 208-247	5 199-248	27 199-254	
		228 16	231 18	227 18	227 17	
Igoumenitsa-2	3 220-228	4 216-248	3 223-245	1 238	11 216-248	
	225 4	235 14	234 11		232 10	
Rana esculenta						
Zagreb-1	7 229-294	10 245-276	0	0	17 229-294	
	264 24	260 12			262 17	
Zagreb-2	8 230-289	7 255-302	0	2 281-338	17 230-338	
	262 19	280 15		310 40	275 24	
San Donà-1	2 228-235	15 211-271	1 232	9 210-259	30 210-271	
	232 5	245 18		238 16	240 16	
San Donà-2	2 232-239	22 238-296	0	18 232-283	42 232-296	
	236 5	262 16		261 14	260 16	
Italian hybrid						
Lavello-1	2 242-248	5 228-259	2 201-227	2 233-238	11 201-259	
	245 4	237 12	214 18	236 4	234 14	
Lavello-2	14 213-258	10 212-268	9 213-262	2 211-241	36 211-268	
	234 15	238 18	236 20	226 21	236 17	
Tarsia-1	2 225-234	19 202-250	2 215-241	10 219-242	33 202-250	
	230 6	224 13	228 18	230 8	226 11	
Tarsia-2	7 220-275	14 203-240	5 220-300	3 216-227	29 203-300	
	241 20	225 10	240 34	222 6	231 19	
Catania-1	12 190-246	16 214-264	11 206-249	17 214-266	58 190-266	
	221 16	240 14	220 13	239 13	231 17	
Catania-2	2 199-207	16 192-234	0	13 189-256	31 189-256	
	203 6	214 12		216 17	214 14	

Table 16. Regional differences of erythrocyte size within taxa.

Mean erythrocyte areas (in μm^2 , calculated from 10 erythrocytes per individual) of adults. The two replicate samples per region are pooled for all taxa with sufficient regional sample sizes. N = number of individuals. SD = empirical standard deviation. Significance level of differences between means of regions: ** = at $p < .01$, *** = at $p < .001$, n = insignificant ($p \geq .05$) (Mann-Whitney U test).

Taxon Region	N	Range	mean	SD	Significance level				
					1	2	3	4	5
Rana ridibunda									
1 Zagreb	19	220-333	266	30	-	n	n	**	***
2 Metković	24	214-300	252	23		-	n	n	**
3 Skadarsko Jezero	7	235-275	249	15			-	n	**
4 Skopje	54	203-281	242	18				-	**
5 Igoumenitsa	13	192-245	226	14					-
					7	9			
Italian nonhybrid									
7 Lavello	19	181-246	218	17	-	n			
9 Catania	11	210-261	232	14		-			
					3	5			
Balkan taxa									
3 Skadarsko Jezero	21	205-284	247	24	-	**			
5 Igoumenitsa	27	199-254	229	16		-			
					1	6	7	8	9
Hybridogenetic hybrids									
Rana esculenta									
1 Zagreb	19	245-338	273	22	-	**	***	***	***
6 San Donà	64	210-296	254	18		-	***	***	***
Italian hybrid									
7 Lavello	19	211-268	236	16			-	**	n
8 Tarsia	46	202-250	225	11				-	n
9 Catania	62	189-266	228	19					-

Table 17. Differences in erythrocyte size between sympatric taxa.

Mean erythrocyte areas (in μm^2 , calculated from 10 erythrocytes per individual) of adults. N = number of individuals; SD = empirical standard deviation; p = probability for insignificant intergroup difference (Mann-Whitney U test).

First taxon Second taxon Locality	First taxon				Second taxon				p
	N	range	mean	SD	N	range	mean	SD	
Rana ridibunda versus Rana esculenta:									
Zagreb-1	10	220-272	249		18	10 245-276	260	12	.17
Zagreb-2	9	250-333	285		31	9 255-338	287	23	.43
Rana ridibunda versus Yugoslavian taxon:									
Skadarsko Jezero-1	3	235-246	242		6	17 205-284	247	26	.63
Skadarsko Jezero-2	4	240-275	255		18	4 228-259	246	13	.56
Rana ridibunda versus Greek taxon:									
Igoumenitsa-1	2	241-245	243		3	22 199-254	228	16	.12
Igoumenitsa-2	11	192-238	223		13	5 216-248	235	12	.09
Italian nonhybrid versus Italian hybrid:									
Lavello-1	16	181-246	217		18	7 228-259	237	10	.01
Catania-1	11	210-261	232		14	33 214-266	239	14	.10

Table 18. Intertaxon differences in erythrocyte size.

Mean erythrocyte areas (in μm^2 , calculated from 10 erythrocytes per individual) of adults. Localities are pooled. N = number of individuals. SD = empirical standard deviation. Significance level of intergroup differences: ** = at $p < .01$, *** = at $p < .001$, n = insignificant ($p \geq .05$) (Mann-Whitney U test).

Taxon	N	range	mean	SD	Significance level					
					1	2	3	4	5	6
1 Rana ridibunda	117	192-333	247	24	-	***	n	***	***	***
2 Italian nonhybrid	32	181-261	223	17		-	**	n	***	n
3 Yugoslavian taxon	21	205-284	247	24			-	**	n	**
4 Greek taxon	27	199-254	229	16				-	***	n
5 Rana esculenta	83	210-338	259	21					-	***
6 Italian hybrid	127	189-268	228	16						-

taxon discussed, the interregional differences often are significant (Table 16).

In comparisons of sympatric taxa at a site (Table 17), Rana ridibunda differed insignificantly from either the Yugoslavian or the Greek taxon. Rana esculenta had slightly larger erythrocytes than sympatric Rana ridibunda (Zagreb), although the difference is not significant. Italian hybrids had somewhat larger erythrocytes than the syntopic nonhybrids, the difference being significant for Lavello-1; slightly longer erythrocytes of hybrids have already been reported for several localities of peninsular Italy (Uzzell and Hotz 1979).

For comparing all taxa, means of erythrocyte areas were pooled for adults of each taxon discussed (Table 18). The resultant group means are largest for Rana esculenta, intermediate for Rana ridibunda and the Yugoslavian taxon, and smallest for the Greek taxon, the Italian hybrid and the Italian nonhybrid; most of these intertaxon differences are highly significant.

All differences in erythrocyte size between means for sexes, age classes, regions and taxa reported above are small, compared to the 1.3 to 1.5-fold differences in mean erythrocyte areas found between sympatric diploid and triploid individuals of central European Rana esculenta lineages (for example Günther 1977). Moreover, no evidence of bimodality was seen, with three possible exceptions, among the local samples discussed. These data thus indicate that mainly diploids are contained in the samples discussed here.

The exceptions are three hybrids with erythrocytes that appeared, within samples, to be outstandingly large. One adult male Rana esculenta from Zagreb-2 had a mean erythrocyte area of $338 \mu\text{m}^2$, which is 20 % larger than that of the second adult male, and than the mean for all other 8 adult hybrids in the sample ($281 \mu\text{m}^2$, ranges 255-302, standard deviation 14). Two immature Italian hybrids from Tarsia-2, one male and one female, had a mean erythrocyte area of 300 and $275 \mu\text{m}^2$, respectively; this is 25 % and 15 % larger than the local mean for the other 4 and 6 immature hybrids of the respective sex (225 and $235 \mu\text{m}^2$, ranges 220-233 and 220-260, standard deviations 6 and 15). For central Europe, Günther (1977) reported erythrocyte sizes for adult water frogs of known karyotype: means of mean areas in triploid compared to diploid Rana esculenta averaged 32 % larger for females, 45 % larger for males. Although these average differences are larger than

those reported for the three hybrids discussed here, the smallest individual mean value of Rana esculenta reported by Günther for triploids was only 17 % (males) and 20 % (females) larger than the largest individual mean for diploids, which is comparable to the values found for the Zagreb and Tarsia individuals. On the basis of erythrocyte size, it cannot therefore be excluded that these three hybrids, especially the two males, are triploid.

Electrophoresis provides another indirect means to estimate ploidy in water frog hybrids. In case of heterozygote electrophoretic phenotypes for some proteins, a gene dosage effect can be detected in triploids, the bands corresponding to the parental allele that is present twice being distinctly more intense than those corresponding to the single other parental allele. Readily visible gene dosage has been demonstrated for triploid central European Rana esculenta by Uzzell, Berger and Günther 1975 (for LDH-1, PGM-2), Günther and Hähnel 1976 (LDH-1), Uzzell, Günther and Berger 1977 (LDH-1), Hemmer 1977 (Alb), Günther, Uzzell and Berger 1979 (LDH-1, PGM-2), Tunner 1979, 1980 (Alb) and Ebendal and Uzzell 1982 (Alb); gene dosage was not visualized in triploids heterozygous for AAT-1, α -GDH and GPI (see Uzzell, Berger and Günther 1975; Uzzell, Günther and Berger 1977; Günther, Uzzell and Berger 1979). A marked gene dosage effect was also detected in allotriploid hybrids of eastern Palearctic water frogs, for LDH-1, an MDH locus and IDH-1 (Nishioka, Ueda and Sumida 1981 a).

Electrophoretic phenotypes of the three hybrids with large erythrocytes were reexamined for evidence of gene dosage. The female from Tarsia, heterozygous 9 times for the 20 protein loci discussed, showed no evidence for gene dosage at any of these 9 loci; this suggests it is diploid.

The male from Tarsia, with still larger erythrocytes, is heterozygous 11 times among the loci studied (AAT-1 b/e, CK a/b, α -GDH a/b, GPI a/d, LDH-1 c/e, MPI c/i, Pep-2 b/c, PGM-2 b/c, 6PGDH b/i, MProt1 b/c, Alb a/i). For 5 of these loci, the electromorph corresponding to the Italian non-hybrid allele was more intense, relative to the ridibunda electromorph, than in the heterozygous patterns seen for these loci on the same gels for other hybrids from the same region (especially CK a and PGM-2 c, but also α -GDH b, MPI i and Alb a); no contrary evidence was seen in any of the other heterozygous loci examined. This suggests, in accord with the data on erythrocyte size, that this male is triploid, and it suggests that two genomes from the Italian nonhybrid and one ridibunda genome are present.

The second possibly triploid male, from Zagreb, is heterozygous 9 times among the loci studied (AAT-1 e/g, α -GDH a/b, IDH-1 a/b, LDH-1 b/c, MPI c/h, Pep-2 b/c, PGM-2 c/d, 6PGDH e/possibly b, Alb a/b). For 2 of these loci, the electromorph corresponding to the ridibunda allele (Pep-2 b, PGM-2 d) was more intense, relative to the lessonae electromorph, than in the heterozygous patterns seen for these loci on the same gels for other Rana esculent from the same region. For the other 7 heterozygous loci no clear gene dosage effect was seen; the lessonae electromorph of one of them (Alb a) was even more intense than the ridibunda electromorph, which, however, is generally observed in the other Rana esculenta also (Appendix 2). These data thus suggest, although with less certainty than for the Tarsia specimen, that this male is a triploid, and, if so, that it contains two genomes of Rana ridibunda and one of Rana lessonae.

APPENDIX 4

ELECTROPHORESIS OF SOMA AND GERM LINE OF HYBRIDS:
HYBRIDOGENESIS AND IDENTIFICATION OF PARENTAL GENOMES

The hybridogenetic genome exclusion in the germ line of water frog hybrids can be detected electrophoretically in gonadal samples (Vogel and Chen 1976; Vogel 1977; Graf, Karch and Moreillon 1977; Uzzell, Hotz and Berger 1980; Tunner 1980; Tunner and Heppich 1981; Heppich, Tunner and Greilhuber 1982; Hotz and Uzzell 1983) and in individual primary oocytes (Uzzell, Hotz and Berger 1980; Tunner 1980; Tunner and Heppich 1981; Hotz and Uzzell 1983): for somatically heterozygous loci, only one allele (usually that of Rana ridibunda) is expressed in normal gametocytes of the hybrids, if they are hybridogenetic.

Electrophoretic comparisons of somatic tissues and germ line cells of hybrids in the samples discussed here were carried out for each region. The results are summarized in Tables 19 (for females) and 20 (for males). In each hybrid examined from each region, for each locus that was somatically heterozygous and scorable in oocytes or testis extracts, the gonadal samples showed only a single allelic product (or occasionally one allelic product predominating in a faintly heterozygous pattern, probably due to somatic tissues of the testes or on the oocyte surfaces). The allelic product exhibited in gonads is known in most cases, across all regions, to characterize Rana ridibunda (Tables 19, 20; see Appendix 2). These data thus confirm that in each region (Zagreb, San Donà, Lavello, Tarsia, Catania) hybrids do reproduce via hybridogenetic gametogenesis, and that in each case (including the hybrids from Zagreb, syntopic with Rana ridibunda) it is the ridibunda genome that is clonally inherited by progeny, and the lessonae or Italian nonhybrid genome that is excluded in gametogenesis.

The results presented in Tables 19 and 20 can be used to assign alleles of somatically heterozygous hybrids to parental genomes as well as to demonstrate hybridogenesis: of two alleles present in the soma, the one expressed in gonads is assumed to belong to the ridibunda genome. This assumption appears justified, because the majority of alleles expressed in gonads of hybrids are known to characterize Rana ridibunda, and because no

Table 19. Electrophoretic comparison of soma and primary oocytes of hybrid females. Electrophoretic phenotypes comprising 7 enzyme loci of somatic tissues and of individual primary oocytes of hybrids from all regions discussed. Only loci somatically heterozygous for an individual are included. FN = field number; n = number of oocytes examined per locus; * = subadult female. ">" indicates relative intensity of electromorphs. In somatic phenotypes the allelic product of the ridibunda genome is indicated by ○, if its allele characterizes Rana ridibunda populations and/or if the other allele characterizes the syntopic non-ridibunda parental species. See Appendix 2 for electrophoretic phenotypes in quotation marks.

Locality	FN	Locus	Alleles expressed		
			Soma	Oocytes	n
Zagreb-1	409	GPI	(a) + d	a >> d	8 (4 × 2 pooled)
		LDH-1	b + (c)	c	8 (4 × 2 pooled)
		Pep-2	(b) + c	b	8 (4 × 2 pooled)
	410	GPI	(a) + d	a >> d	5 (pooled)
		LDH-1	(c) + e	c	5 (pooled)
Zagreb-2	432	GPI	(a) + d	a	4
		LDH-1	(c) + e	c	4
		MPI	(c) + h	c	4
		Pep-2	(b) + c	b	4
	458	GPI	d + f	d	36 (8 × 2 pooled)
		LDH-1	b + (c)	c	28 (4 × 2 pooled)
		MPI	(c) + h	c	8 (4 × 2 pooled)
		Pep-2	(b) + c	b	2 (pooled)
San Dona-1	925	GPI	(a) + d	a	8
		LDH-1	(c) + e	c	4
		MPI	(c) + h	c	4
		Pep-2	(b) + c	b	4
	933	GPI	(a) + d	a	8
		LDH-1	b + (c)	c	4
		MPI	(c) + h	c	4
		Pep-2	(b) + c	b	4
	946 *	GPI	(a) + d	a > d	14 (pooled)
		LDH-1	(c) + e	c > e	14 (pooled)
		MDH-1	(b) + c	b >> c	14 (pooled)
Lavello-1	836	AAT-1	e + j	j	8 (2 × 2 pooled)
		GPI	(a) + d	a	14 (5 × 2 pooled)
		LDH-1	(c) + e	c	20 (8 × 2 pooled)
		MPI	(c) + i	c	8 (2 × 2 pooled)
	846	Pep-2	(b) + c	b	6 (pooled)
		GPI	(a) + d	a	3
		LDH-1	(c) + f	c	3

Table 19 (continued).

Locality	FN	Locus	Alleles expressed		
			Soma	Oocytes	n
Lavello-2	900	AAT-1	e + j	j	4
		GPI	(a) + d	a	8
		LDH-1	(c) + e	c	8
		MPI	(c) + i	c	4
		Pep-2	(b) + c	b	4
Tarsia-1	779	AAT-1	e + h	e	3
		GPI	(a) + d	a	4
		LDH-1	(c) + e	c	4
		MPI	(c) + f	c	4
	783	AAT-1	d + (e)	e	3
		GPI	(a) + d	a	4
		LDH-1	(c) + f	c	4
		MPI	(c) + i	c	4
Tarsia-2	817	AAT-1	b + e	e	13
		GPI	(a) + d	a	27
		LDH-1	(c) + e	c	31
		MDH-1	(b) + c	b	18
		MPI	(c) + i	c	17
		Pep-2	(b) + c	b	8
	818	6PGDH	"a" + (d)	d	8
		6PGDH	b + (d)	d	6
	822	GPI	(a) + d	a	14
		LDH-1	(c) + e	c	16
		MPI	(c) + f	c	8
		Pep-2	(b) + c	b	8
	829	GPI	(a) + d	a	12
		LDH-1	(c) + e	c	16
Catania-1	720	GPI	(a) + d	a	2
		LDH-1	(c) + e	c	3
		MDH-1	(b) + c	b	3
		Pep-2	(b) + c	b	3
Catania-2	748	GPI	(a) + d	a	12
		LDH-1	(c) + e	c	20
	763	GPI	(a) + d	a	8
		LDH-1	(c) + e	c	10
		MDH-1	(b) + c	b	9
		MPI	"(c) + f"	c	5
	768	GPI	(a) + d	a	10
		LDH-1	(c) + e	c	20

Table 20. Electrophoretic comparison of soma and testis extracts of hybrid males. Electrophoretic phenotypes comprising 5 enzyme loci of somatic tissues and of testis extracts of hybrids. Only loci somatically heterozygous for an individual are included.

FN = field number. * = possibly triploid with 2 ridibunda and 1 lessonae genomes; ** = possibly triploid with 1 ridibunda and 2 Italian nonhybrid genomes (see Appendix 3). ">" indicates relative intensity of electrophoretic bands. In somatic phenotypes the allelic product of the ridibunda genome is indicated by ○, if its allele characterizes Rana ridibunda populations and/or if the other allele characterizes the syntopic non-ridibunda parental species. See Appendix 2 for electrophoretic phenotypes in quotation marks.

Locality	FN	Locus	Alleles expressed	
			Soma	Testes
Zagreb-2	435 *	Pep-2 6PGDH	ⓑ + c "b" + ⓔ	b >> c e
	461	6PGDH	"b" + ⓓ	d
San Dona-1	928	6PGDH	"b" + ⓓ	d
	936	AAT-1 LDH-1	ⓔ + i b + ⓐ	e c
	940	6PGDH	"b" + ⓔ	e
San Dona-2	951	6PGDH	"b" + ⓔ	e >> "b"
	970	6PGDH	"b" + ⓔ	e >> "b"
Lavello-1	856	AAT-1 GPI LDH-1	e + j ⓐ + d ⓐ + e	j a c
	868	6PGDH	"b" + ⓔ	e
Lavello-2	919	6PGDH	"b" + ⓓ	d
Tarsia-2	807	LDH-1	ⓐ + e	c >> e
	810	LDH-1	ⓐ + e	c >> e
	824 **	GPI	ⓐ + d	a
	834	LDH-1	ⓐ + e	c >> e
Catania-2	769	LDH-1	ⓐ + e	c

evidence for recombination in their hybrid ancestors was detected in any of the hybrids discussed (not a single hybrid homozygous at any locus for an allele characterizing the syntopic non-ridibunda parental species was found); see however Appendix 5 for a discussion of the population system at the two Zagreb sites differing from the Italian samples by the syntopy of hybrids with Rana ridibunda.

Among the 14 loci examined that were generally or occasionally heterozygous in the hybridogenetic hybrids discussed, 17 out of a total of 56 alleles observed in hybrids are clearly assignable to ridibunda genomes, because they are known to characterize present-day Rana ridibunda populations, or because, at a locus, all other alleles found to cooccur with them in heterozygous hybrids are characteristic for the syntopic non-ridibunda parental species (see Appendix 2). These ridibunda alleles are CK b; α -GDH a; GPI a; LDH-1 c; MDH-1 b; MPI a, c; Pep-2 b; PGM-2 b, d; 6PGDH d, e; MProt1 b; Alb b, c, h, i; and, in addition, AAT-1 e (except in b/e, e/h, e/j heterozygotes) and IDH-1 b (except in a/b heterozygotes); Similarly, 29 other alleles observed in hybrids are clearly assignable to the non-ridibunda genomes, because they characterize populations of the syntopic non-ridibunda parental species, or because, at a locus, all other alleles found to cooccur with them in heterozygous hybrids are characteristic for Rana ridibunda. These non-ridibunda alleles are AAT-1 a, d, g, j; CK a; α -GDH b, c; GPI g; IDH-1 c; LDH-1 b, e, f; MDH-1 c; MPI f, g, h, i; Pep-2 c, d; PGM-2 c, e, f; 6PGDH a, b, c; MProt1 c; Alb a, f, g; and, in addition, GPI d, f (both except in d/f heterozygotes at Zagreb). The parental genome assignment of 10 alleles observed in hybrids remains uncertain by these criteria: AAT-1 b, h, j and e (in b/e, e/h, e/j heterozygotes); GPI d, f (in d/f heterozygotes at Zagreb); IDH-1 a and b (in a/b heterozygotes); IDH-2 a and b (in a/b heterozygotes).

The results of gonad electrophoresis in Tables 19 and 20 abundantly confirm the proposed assignments of hybrid alleles to parental genomes for the ridibunda alleles AAT-1 e (San Donà, Tarsia), GPI a (each region), LDH-1 c (each region), MDH-1 b (San Donà, Tarsia, Catania), MPI c (each region), Pep-2 b (each region), 6PGDH d (Zagreb, San Donà, Lavello, Tarsia) and e (Zagreb, San Donà, Lavello); and for the non-ridibunda alleles AAT-1 d (Tarsia) and j (San Donà), GPI d (each region), LDH-1 b (Zagreb, San Donà), e (each region) and f (Lavello, Tarsia), MDH-1 c (San Donà, Tarsia, Catania), MPI f (Tarsia, Catania: see Appendix 2 for phenotype), h

(Zagreb, San Donà) and i (Lavello, Tarsia), Pep-2 c (each region), 6PGDH "a" (Tarsia), b (Tarsia) and "b" (Zagreb, San Donà, Lavello; see Appendix 2 for quotation marks). These non-ridibunda alleles include those only found in hybrids at low frequency in some regions (MDH-1 e in female 946 from San Donà; AAT-1 d in female 783 from Tarsia and i in male 936 from San Donà; LDH-1 f in females 846 from Lavello and 783 from Tarsia).

Among the 10 cases of alleles in hybrids not clearly assignable to parental genomes by their patterns of occurrence in somatic tissues, 4 (IDH-1 a and b in a a/b heterozygote each from Zagreb and Tarsia, IDH-2 a and b in a a/b heterozygote from San Donà) are not assignable by electrophoresis of gonad extracts either, because neither IDH locus was sufficiently active in oocytes or testis extracts to be scored. Although subjectively it seems more likely that the rare IDH-1 a and IDH-2 a alleles belong to the syntopic non-ridibunda parental species rather than representing separate ridibunda clones (for IDH-1 a at Tarsia and IDH-2 a at San Donà, outside of the Rana ridibunda range), no conclusive evidence is available; these alleles are therefore used to estimate individual heterozygosity, but not to estimate frequency of polymorphic loci and number of alleles per locus.

For GPI, female 458 from Zagreb-2, a d/f heterozygote, exhibited only the d allelic product in all oocytes examined (Table 19), demonstrating that the rare f allele belongs to the lessinae genome (analogous to LDH-1 b, MPI h, Pep-2 c of the same individual); this is consistent with the known f and d allele distributions (Appendix 2), but was not a priori expected because f also occurs in Balkan Rana ridibunda, although not in the Zagreb samples.

For AAT-1, 2 alleles of hybrids (b, h, both found at Tarsia) are not clearly assignable to parental genomes, because they were not observed in Italian nonhybrids and because in the soma they occurred together with the allele e that is shared by ridibunda genomes and the Italian nonhybrid. In both cases, only the e allele was expressed in gonads (females 817 and 779, Table 19), demonstrating that b and h are Italian nonhybrid alleles. That the i allele of Lavello, although never found so far in Rana ridibunda populations, nevertheless belongs to ridibunda genomes is suggested (1) by its exclusive occurrence in hybrids (at Lavello found heterozygous in 12 of 47 hybrids, not found among the 37 nonhybrids examined), and (2) by one hybrid from Lavello-2 that is a d/i heterozygote, neither of the two alleles being known for ridibunda genomes, but the d allele occurring in the local

Italian nonhybrid samples. This conclusion is confirmed by two females (836, 900; Table 19) and one male (856; Table 20), all $\underline{e}/\underline{j}$ heterozygotes, evidencing only the $\underline{j}$ allelic product in gonads.

APPENDIX 5

POPULATION SYSTEMS OF HYBRID LINEAGES

Because the validity of any conclusions on genetic variation in the water frog hybrids studied depends on basic knowledge about their population structure and mode of reproduction, this background information is summarized below; supporting evidence is briefly discussed.

- (1) Hybrids constitute hemiclinal hybridogenetic lineages and inherit their ridibunda genomes.

That the electrophoretically identified interspecific hybrids in the Italian region reproduce, like central European Rana esculenta, via hybridogenetic gametogenesis is already shown by their geographic distribution: Throughout this area, they live syntopically with their non-ridibunda parental species (Rana lessonae in the Po valley drainage area, the Italian nonhybrid in peninsular Italy and on Sicily), but outside of the present Rana ridibunda range (Uzzell and Hotz 1979; see also data in Table 14 and Fig. 3 p.). Although this evidence is indirect, no alternative explanation for the electrophoretic data revealing their ridibunda genome is available (Uzzell and Hotz 1979). Direct demonstration of hybridogenesis is provided by electrophoretic comparison of soma and germ line cells of hybrids (Appendix 4). Such evidence has previously been reported for 4 water frog hybrids from Italy without locality data (Tunner 1980), and for 3 hybrids from the Tarsia samples of the present study (Hotz and Uzzell 1983). Hybridogenesis has also been directly demonstrated for hybrids from Sicily by crossing experiments (Berger, Uzzell and Hotz 1982 and unpublished). For the samples discussed here, the electrophoretic results presented in Appendix 4 demonstrate that in each region studied (Zagreb, San Donà, Lavello, Tarsia, Catania) hybrids do reproduce via hybridogenetic gametogenesis, and that it is, in each case examined, the ridibunda genome that is clonally transmitted to progeny, and the non-ridibunda parental genome that is excluded in the germ line.

(2) Most hybridogenetic hybrids discussed are somatically diploid.

Some Rana esculenta of both sexes produce, usually in low frequencies, diploid gametes containing in most cases both parental genomes that lead to triploid progeny when fertilized by haploid gametes (Uzzell, Berger and Günther 1975; Uzzell, Günther and Berger 1977). Triploids, of both sexes, have been reported within Rana esculenta lineages in natural populations of central Europe, most numerous along the northern margin of the Rana esculenta range (Wickbom 1945; Günther 1970, 1973, 1975 a,b, 1977; Uzzell and Berger 1975; Uzzell, Berger and Günther 1975; Knudsen and Scheel 1975; Günther and Hähnel 1976; Hemmer 1977; Berger, Roguski and Uzzell 1978; Berger and Roguski 1978; Günther, Uzzell and Berger 1979; Berger 1979; Ebendal and Uzzell 1982). These triploids have either two ridibunda genomes and one lessonae genome or vice versa; they apparently pass the set that is present twice in the soma to haploid gametes (Günther, Uzzell and Berger 1979). Because of these findings, I determined ploidy of the samples discussed, using erythrocyte size and partly electrophoresis. The results are presented in Appendix 3: Probably only two triploids (0.7 % of the 304 hybridogenetic hybrids studied) are contained in these samples. An immature male Italian hybrid from Tarsia-2 probably is a triploid with one ridibunda genome and two Italian nonhybrid genomes. It seems most plausible that it arose via a diploid ovum of a hybrid mother, containing both parental genomes, that was fertilized by a nonhybrid sperm (in analogy to central European Rana esculenta: Uzzell, Berger and Günther 1975; Uzzell, Günther and Berger 1977). A haploid nonhybrid ovum fertilized by a diploid hybrid sperm is also possible but seems less likely (given the lower frequency and reduced fertility of male hybrids reported below, and given the larger body size of hybrids and the preferential mating of water frog males with larger females); and also the fertilization of a haploid hybrid gamete by a diploid nonhybrid gamete cannot be excluded. It is interesting that this probable triploid evidenced only the ridibunda allelic product of one locus examined in a testis extract (GPI a: Table 20 in Appendix 4), suggesting germ line exclusion of the parental genomes present twice in the soma, contrary to central European Rana esculenta triploids (Günther, Uzzell and Berger 1979). An adult male Rana esculenta from Zagreb-2 is the second possible triploid, with two ridibunda genomes and one lessonae genome. If so, it seems likely that it arose via fertilization of a diploid Rana esculenta ovum (most hybrids at Zagreb are females) that contained both parental genomes by a haploid sperm of the sympatric Rana ridibunda (at none of the 9 loci examined that

vary locally in Rana ridibunda were two different ridibunda alleles observed in this hybrid). In accord with triploid Rana esculenta of central Europe having the same genomic composition, its ridibunda allele is expressed in testis extracts for 2 loci examined (Pep-2 b, 6PGDH e: Table 20 in Appendix 4).

(3) Hybrids in Italy live in mixed nonhybrid/hybrid population systems.

These diploid hybridogenetic hybrids coexist throughout the Italian region with their non-ridibunda parental species (Table 14, Fig. 3), outside of the present autochthonous Rana ridibunda range (Fig. 1; see Hotz 1974, Uzzell and Hotz 1979, Hotz and Bruno 1980). They therefore form population systems that are analogous to the central European lessonae-esculenta (L-E) system (Uzzell and Berger 1975): the hybrids live with and, because of hybridogenesis, reproductively depend on Rana lessonae or the Italian non-hybrid to replace the chromosome set lost during gametogenesis. That at some sites only hybrids were contained in larger samples (29 at Tarsia-2, 31 at Catania-2, and data in Table 14, Fig. 3) probably reflects the higher frequency of hybrids in disturbed or artificial habitats (Uzzell and Hotz 1979; compare Appendix 1), or migrants from mixed populations (Uzzell and Hotz 1979). Analogous relative abundances of hybrids are known for central European Rana esculenta in L-E system populations (Lac 1959 a,b; Berger 1964, 1966; Günther 1970, 1973, 1975 a; Juszczuk 1971; Tunner 1972; Escher 1972; Blankenhorn, Heusser and Notter 1973; Blankenhorn 1973, 1974; Tunner and Dobrowsky 1976).

In almost all hybrid samples females outnumber males (Table 2 p. 11; locally 50-72 %, in sum 61 % of 264 hybrids from Italy discussed are females). This can be explained by hybridogenesis and by male heterogamety: no male-determining factors are contained in the clonally inherited ridibunda genomes of hybrids, because primary hybridizations occur between females of the large Rana ridibunda and males of the small Rana lessonae, the reverse being unlikely for behavioral reasons (males mate with larger females; for example Blankenhorn 1974). Given hybridogenesis and male heterogamety, an approximately 1:1 sex ratio is therefore expected in offspring from hybrid female × nonhybrid male matings, whereas all-female progeny is expected from nonhybrid female × hybrid male matings. This has been empirically confirmed for Italian hybrids from Sicily (Berger, Uzzell and Hotz 1982). Males

of the Italian hybrid, like Rana esculenta males (Berger 1967, 1970, 1971 b, 1973 c, 1976; Günther 1973), show somewhat reduced fertility (Berger, Uzzell and Hotz 1982 and unpublished) and possibly do not mate frequently with the often small (Uzzell and Hotz 1979) nonhybrid females; the presence of both sexes in natural population of hybrids indicates that these lineages persist mainly through female hybrid $\times$ male nonhybrid matings (Berger, Uzzell and Hotz 1982), like Rana esculenta in the L-E system. Nevertheless, the all-female progeny of each successful nonhybrid female $\times$ hybrid male mating will bias the hybrid sex ratio in a population in favor of females.

(4) Hybrids at the Zagreb sites are syntopic with Rana ridibunda, but reproduce like L-E system Rana esculenta.

At both Zagreb sites, Rana esculenta occurred in a peculiar population system, different from that of the Italian localities and from all those so far reported for central Europe. (a) The hybrids were syntopic with Rana ridibunda (equally frequent as hybrids: Table 1) and with Rana lessonae (rare: only 1 = 3 % at Zagreb-2). (b) Almost all hybrids collected are females (Table 2; 100 % at Zagreb-1, 88 % at Zagreb-2, one of the only two males possibly a triploid: Appendix 3). (c) In each case examined, the ridibunda alleles of hybrids were shown to be passed to gametes (4 females and 2 males, for 16 somatic heterozygosities at 5 enzyme loci: Tables 19 and 20 in Appendix 4).

Populations that consist of Rana ridibunda and Rana esculenta are also found in central Europe, mainly along the northern margin of the Rana esculenta area (R-E system: Uzzell and Berger 1975). One has been intensively studied (Uzzell, Günther and Berger 1977): virtually all Rana esculenta were diploid males, hybridogenetic, some passing only their lessonae genome to progeny, others only the ridibunda genome, others yet, sometimes the lessonae, sometimes the ridibunda genome; in progeny of 13 crosses, lessonae genomes were inherited about twice as often as ridibunda genomes. The Zagreb populations clearly are not R-E systems in this sense: The prevailing sex of hybrids at Zagreb is female instead of male, and gametogenesis in all hybrids examined is exclusively of the L-E system type. Mixed populations of Rana ridibunda and Rana esculenta that apparently do not form a R-E system occasionally exist in central Europe also (see Günther 1968, 1973, 1974, 1975 a; Blankenhorn 1973; Feldmann and Preywisch 1973; L. Berger, personal

communication). The nearly all-femaleness of Rana esculenta at Zagreb is in accord with the presently unexplained data reported for Rana esculenta populations (mainly of the L-E system) in other parts of the Danube drainage area (Tunner 1974, 1980, Tunner and Dobrowsky 1976), the hybrids persisting through Rana esculenta × Rana lessonae matings that produce mainly female offspring (Tunner 1980; Berger, Uzzell and Hotz unpublished). The two Zagreb localities, both streams with running water, were sampled in August (and September). It is likely that either the rarity of Rana lessonae reflects its terrestrial life during the summer, already reported for the same region of Yugoslavia (Karaman 1948) and for central Europe (Lăc 1959 b, Günther 1968, Berger 1970); or that the local samples collected do not represent a breeding population, but reflect post-breeding immigration of Rana esculenta from a mixed L-E system breeding deme into an estival habitat (high migration capacity of Rana esculenta has been reported by Berger 1970, 1973 a, Heym 1974, Tunner and Dobrowsky 1976). The water frog population structure of the Zagreb sites needs further investigation.

LITERATURE CITED

- Angus, R. A. (1980): Geographic dispersal and clonal diversity in unisexual fish populations. *American Naturalist* 115 (4) : 531-550.
- Angus, R. A. and R. J. Schultz (1979): Clonal diversity in the unisexual fish Poeciliopsis monacha-lucida: a tissue graft analysis. *Evolution* 33 (1,1) : 27-40.
- Berger, L. (1964): Is Rana esculenta lessonae Camerano a distinct species? *Annales zoologici* 22 (13) : 245-261.
- Berger, L. (1966): Biometrical studies on the population of green frogs from the environs of Poznań. *Annales zoologici* 23 (11) : 303-324.
- Berger, L. (1967): Embryonal and larval development of F₁ generation of green frogs different combinations. *Acta zoologica cracoviensia* 12 (7) : 123-160.
- Berger, L. (1968): Morphology of the F₁ generation of various crosses within Rana esculenta complex. *Acta zoologica cracoviensia* 13 (13) : 301-324.
- Berger, L. (1970): Some characteristics of crosses within Rana esculenta complex in postlarval development. *Annales zoologici* 27 (17) : 373-416.
- Berger, L. (1971 a): Sex ratio in the F₁ progeny within forms of Rana esculenta complex. *Genetica polonica* 12 (1-2) : 87-101.
- Berger, L. (1971 b): Viability, sex and morphology of F₂ generation within forms of Rana esculenta complex. *Zoologica Poloniae* 21 (4) : 345-393.
- Berger, L. (1973 a): Systematics and hybridization in European green frogs of Rana esculenta complex. *Journal of Herpetology* 7 (1) : 1-10.
- Berger, L. (1973 b): Some characteristics of backcrosses within forms of Rana esculenta complex. *Genetica polonica* 14 (4) : 413-430.
- Berger, L. (1973 c): Sexual maturity of males within forms of Rana esculenta complex. *Zoologica Poloniae* 22 (3) : 177-188.
- Berger, L. (1976): Hybrids of B₂ generation of European water frogs (Rana esculenta complex). *Annales zoologici* 33 (12) : 201-214.
- Berger, L. (1977): Systematics and hybridization in the Rana esculenta complex. In: Taylor, D. H. and S. I. Guttman (Ed.): *The reproductive biology of amphibians*. Plenum Press, New York and London : 367-388.
- Berger, L. (1979): Egg size as an index of phenotype in progeny of Rana esculenta females. *Mitteilungen aus dem zoologischen Museum in Berlin* 55 (1) : 187-202.

- Berger, L. (1983): Western Palearctic water frogs (Amphibia, Ranidae): Systematics, genetics and population compositions. *Experientia* 39 (2) : 127-130.
- Berger, L. and H. Roguski (1978): Ploidy of progeny from different egg size classes of Rana esculenta L. *Folia biologica*, Kraków, 26 (4) : 231-248.
- Berger, L., H. Roguski and T. Uzzell (1978): Triploid F₂ progeny of water frogs (Rana esculenta complex). *Folia biologica*, Kraków, 26 (3) : 135-152.
- Berger, L. and J. Truszkowski (1980): Viability and inheritance of characters in water frogs (Rana esculenta complex) in agrocenoses. *Genetica polonica* 21 (3) : 309-323.
- Berger, L. and T. Uzzell (1977): Vitality and growth of progeny from different egg size classes of Rana esculenta L. (Amphibia, Salientia). *Zoologica Poloniae* 26 (3-4) : 291-317.
- Berger, L. and T. Uzzell (1980): The eggs of European water frogs (Rana esculenta complex) and their hybrids. *Folia biologica*, Kraków, 28 (1) : 3-25.
- Berger, L., T. Uzzell and H. Hotz (1982): Crossing experiments between some western Palearctic species of water frogs (Salientia: Ranidae). *Vertebrata hungarica* 21 : 33-45 + 2 plates.
- Bezy, R. L. (1972): Karyotypic variation and evolution of the lizards in the family Xantusiidae. *Contributions in Science, Natural History Museum Los Angeles County*, 227 : 1-29.
- Binkert, J., P. Borner and P. S. Chen (1982): Rana esculenta complex: an experimental analysis of lethality and hybridogenesis. *Experientia* 38 (11) : 1283-1292.
- Blankenhorn, H. J. (1973): Zum Stand der Forschung über die Verbreitung der Grünfrösche im Kanton Zürich. *Revue suisse de Zoologie* 80 (3) : 656-661.
- Blankenhorn, H. (1974): Soziale Organisation einer Mischpopulation von Rana lessonae Camerano und Rana esculenta Linnaeus. Dissertation Universität Zürich : 155 pp.
- Blankenhorn, H. J. (1977): Reproduction and mating behavior in Rana lessonae-Rana esculenta mixed populations. In: Taylor, D. H. and S. I. Guttman (Ed.): *The reproductive biology of amphibians*. Plenum Press, New York and London : 389-410.
- Blankenhorn, H. J., H. Heusser and P. Notter (1973): Zur Verbreitung von Rana esculenta Linnaeus und Rana lessonae Camerano im Zürcher Oberland. *Revue suisse de Zoologie* 80 (3) : 662-666.

- Blankenhorn, H. J., H. Heusser and P. Vogel (1971): Drei Phänotypen von Grünfröschen aus dem Rana esculenta-Komplex in der Schweiz. *Revue suisse de Zoologie* 78 : 1242-1247.
- Brown, A. H. D. (1979): Enzyme polymorphism in plant populations. *Theoretical Population Biology* 15 (1) : 1-42.
- Bulger, A. J. and R. J. Schultz (1979): Heterosis and interclonal variation in thermal tolerance in unisexual fishes. *Evolution* 33 (3) : 848-859.
- Bulger, A. J. and R. J. Schultz (1982): Origin of thermal adaptations in northern vs. southern populations of a unisexual hybrid fish. *Evolution* 36 (5) : 1041-1050.
- Cimino, M. C. (1972 a): Egg-production, polyploidization and evolution in a diploid all-female fish of the genus Poeciliopsis. *Evolution* 26 (2) : 294-306.
- Cimino, M. C. (1972 b): Meiosis in triploid all-female fish (Poeciliopsis, Poeciliidae). *Science* 175 (4029) : 1484-1486.
- Cole, C. J. (1975): Evolution of parthenogenetic species of reptiles. In: Reinboth, R. (Ed.): *Intersexuality in the animal kingdom*. Springer-Verlag, Berlin, Heidelberg and New York : 340-355.
- Cole, C. J. (1978): The value of virgin birth. *Natural History* 87 (1) : 56-63.
- Cole, C. J. (1980): Newly attained polyploidy and/or clonal reproduction in animals: the brink of extinction, or threshold to new frontiers? In: Lewis, W. H. (Ed.): *Polyploidy: biological relevance*. Plenum Press, New York and London : 539-542.
- Cuellar, O. (1971): Reproduction and the mechanism of meiotic restitution in the parthenogenetic lizard Cnemidophorus uniparens. *Journal of Morphology* 133 (2) : 139-165.
- Cuellar, O. (1976): Cytology of meiosis in the triploid gynogenetic salamander Ambystoma tremblayi. *Chromosoma* 58 (4) : 355-364.
- Cuellar, O. (1977): Animal parthenogenesis. A new evolutionary-ecological model is needed. *Science* 197 (4306) : 837-843.
- Darevskij, I. S. (1958): Estestvennyi partenogenez u nekotorych podvidov skal'noj jaščericy (Lacerta saxicola Eversmann) rasprostranennykh v Armenii. *Doklady Akademii Nauk SSSR* 122 (4) : 730-732.
- Darevsky, I. S. (1966): Natural parthenogenesis in a polymorphic group of Caucasian rock lizards related to Lacerta saxicola Eversmann. *Journal of the Ohio Herpetological Society* 5 : 115-152.
- Darevsky, I. S. (1967): Rock lizards of the Caucasus (Systematics, ecology, and phylogeny of the polymorphic lizards of the Caucasus of the subge-

- nus Archaeolacerta). Izdatel'stvo Nauka, Leningrad : 214 pp. [English translation by Zug, G. R. (Ed.) (1978), Indian National Scientific Documentation Centre, New Delhi : VI + 276 pp.]
- Dixon, W. J. (Ed.) (1981): BMDP statistical software 1981. University of California Press, Berkeley : X + 726 pp.
- Dobzhansky, T. (1951): Genetics and the origin of species. Third edition. Columbia University Press, New York and London : 364 pp.
- Downs, F. L. (1978): Unisexual Ambystoma from the Bass Islands of Lake Erie. Occasional Papers of the Museum of Zoology University of Michigan 685 : 1-36.
- Dubois, A. (1977): Les problèmes de l'espèce chez les Amphibiens Anoures. In: Bocquet, C., J. Génarmont and M. Lamotte (Ed.), Les problèmes de l'espèce dans le règne animal. Mémoires de la Société zoologique de France 39 : 161-284.
- Ebendal, T. (1976): A Swedish population of Rana lessonae - chromosomal features and relations to the Rana esculenta complex. Norwegian Journal of Zoology 24 : 231-232.
- Ebendal, T. (1977): Karyotype and serum protein pattern in a Swedish population of Rana lessonae (Amphibia, Anura). Hereditas 85 : 75-80.
- Ebendal, T. and T. Uzzell (1982): Ploidy and immunological distance in Swedish water frogs (Rana esculenta complex). Amphibia Reptilia 3 (2/3) : 125-133.
- Eisenbrey, A. B. and W. S. Moore (1981): Evolution of histocompatibility diversity in an asexual vertebrate, Poeciliopsis 2 monacha-lucida (Pisces: Poeciliidae). Evolution 35 (6) : 1180-1191.
- Engelmann, W.-E. (1972): Disk-Elektrophorese der Serumproteine von Wasserfröschen. Ein Beitrag zur Diskussion über den Hybridcharakter von Rana esculenta L. Acta biologica et medica germanica 29 : 431-435.
- Engelmann, W.-E. (1973): Zur Frage der verwandtschaftlichen Beziehungen europäischer Grünfrösche (Gattung Rana). Eine vergleichende elektrophoretische Untersuchung der Serumproteine. Zoologische Jahrbücher (Systematik) 100 (2) : 183-196.
- Escher, K. (1972): Die Amphibien des Kantons Zürich. (Bestand und Lebensbedingungen 1967-1969; Schlüsse für den Amphibienschutz). Vierteljahrsschrift der naturforschenden Gesellschaft in Zürich 117 (Schlussheft) : 335-380.
- Fankhauser, G. (1945): The effects of change in chromosome number on amphibian development. Quarterly Review of Biology 20 : 20-78.

- Feldmann, R. and K. Preywisch (1973): Seefrosch, Wasserfrosch und Kleiner Grünfrosch im Wesertal bei Höxter (Westfalen). *Natur und Heimat* 33 (4) : 120-126.
- Fuhn, I. E., C. Şova and M. Dumitrescu (1975): Une population hybridogène Triturus v. vulgaris L. × T. montandoni Boul. du Lac Crăciurele (Mts. Nemira, Départ. Bacău). *Studii şi Comunicari Muzeul de Ştiinţele Naturii, Bacău*, 8 : 225-236.
- George, S. A. and M. R. Lennartz (1980): Methods for determining ploidy in amphibians: nucleolar number and erythrocyte size. *Experientia* 36 (6) : 687-688.
- Graf, J.-D., F. Karch and M.-C. Moreillon (1977): Biochemical variation in the Rana esculenta complex: A new hybrid form related to Rana perezi and Rana ridibunda. *Experientia* 33 (12) : 1582-1584.
- Graf, J.-D. and W. P. Müller (1979): Experimental gynogenesis provides evidence of hybridogenetic reproduction in the Rana esculenta complex. *Experientia* 35 (12) : 1574-1576.
- Günther, R. (1968): Morphologische und ökologische Untersuchungen zur Unterscheidung von Rana esculenta L. und Rana ridibunda Pall. *Zoologische Jahrbücher (Systematik)* 95 (1-2) : 229-264.
- Günther, R. (1970): Der Karyotyp von Rana ridibunda Pall. und das Vorkommen von Triploidie bei Rana esculenta L. (Anura, Amphibia). *Biologisches Zentralblatt* 89 : 327-342.
- Günther, R. (1973): Über die verwandtschaftlichen Beziehungen zwischen den europäischen Grünfröschen und den Bastardcharakter von Rana esculenta L. (Anura). *Zoologischer Anzeiger* 190 (3/4) : 250-285.
- Günther, R. (1974) : Neue Daten zur Verbreitung und Ökologie der Grünfrösche (Anura, Ranidae) in der DDR. *Mitteilungen aus dem zoologischen Museum in Berlin* 50 (2) : 287-298.
- Günther, R. (1975 a): Zum natürlichen Vorkommen und zur Morphologie triploider Teichfrösche, "Rana esculenta", L., in der DDR (Anura, Ranidae). *Mitteilungen aus dem zoologischen Museum in Berlin* 51 (1) : 145-158.
- Günther, R. (1975 b): Untersuchungen der Meiose bei Männchen von Rana ridibunda Pall., Rana lessonae Cam. und deren Bastardform "Rana esculenta" L. (Anura). *Biologisches Zentralblatt* 94 : 277-294.
- Günther, R. (1977): Die Erythrozytengröße als Kriterium zur Unterscheidung diploider und triploider Teichfrösche, Rana "esculenta" L. (Anura). *Biologisches Zentralblatt* 96 (4) : 457-466.

- Günther, R. and S. Hähnel (1976): Untersuchungen über den Genfluss zwischen Rana ridibunda und Rana lessonae sowie die Rekombinationsrate bei der Bastardform Rana "esculenta" (Anura, Ranidae). Zoologischer Anzeiger 197 (1/2) : 23-38.
- Günther, R. and S. Lübecke (1979): Serological studies in water frogs from the GDR, Bulgaria, and the Soviet Union. Mitteilungen aus dem zoologischen Museum in Berlin 55 (1) : 225-229.
- Günther, R., T. Uzzell and L. Berger (1979): Inheritance patterns in triploid Rana "esculenta" (Amphibia, Salientia). Mitteilungen aus dem zoologischen Museum in Berlin 55 (1) : 35-57.
- Hall, W. P. (1970): Three probable cases of parthenogenesis in lizards (Agamidae, Chamaeleontidae, Gekkonidae). Experientia 26 (11) : 1271-1273.
- Harris, H. and D. A. Hopkinson (1976): Handbook of enzyme electrophoresis in human genetics. North-Holland Publishing Company, Amsterdam and Oxford.
- Hemmer, H. (1977): Studien an einer nordwestdeutschen Grünfroschpopulation als Beitrag zur Bestimmungsproblematik und zur Rolle der Selektion in Rana esculenta-Komplex (Amphibia: Salientia: Ranidae). Salamandra 13 (3/4) : 166-173.
- Hemmer, H., A. Konrad and K. Bachmann (1980): Hybridization within the Rana ridibunda complex of North Africa. Amphibia Reptilia 1 (1) : 41-48.
- Heppich, S., H. G. Tunner and J. Greilhuber (1982): Premeiotic chromosome doubling after genome elimination during spermatogenesis of the species hybrid Rana esculenta. Theoretical and applied Genetics 61 : 101-104.
- Heusser, H. and H. J. Blankenhorn (1973): Crowding-Experimente mit Kaulquappen aus homo- und heterotypischen Kreuzungen der Phänotypen esculenta, lessonae und ridibunda (Rana esculenta-Komplex, Anura, Amphibia). Revue suisse de Zoologie 80 (2) : 543-569.
- Hotz, H. (1974): Ein Problem aus vielen Fragen - europäische Grünfrösche (Rana esculenta-Komplex) und ihre Verbreitung. Natur und Museum 104 (9) : 262-272.
- Hotz, H. and S. Bruno (1980): Il problema delle rane verdi e l'Italia (Amphibia, Salientia). Rendiconti Accademia nazionale delle Scienze detta dei XL, Memorie di Scienze fisiche e naturali, 98 (4) (6) : 49-112.
- Hotz, H. and T. Uzzell (1982): Biochemically detected sympatry of two water frog species: two different cases in the Adriatic Balkans (Amphibia, Ranidae). Proceedings of the Academy of Natural Sciences of Philadelphia 134 : 50-79.

- Hotz, H. and T. Uzzell (1983): Interspecific hybrids of Rana ridibunda without germ line exclusion of a parental genome. *Experientia* 39 (5) : 538-540.
- Hubbs, C. L. and L. C. Hubbs (1932): Apparent parthenogenesis in nature in a form of fish of hybrid origin. *Science* 76 : 628-630.
- Juszczyk, W. (1971): The morphometric structure of population of green frogs from southern Poland. *Acta biologica cracoviensia* 14 : 197-209.
- Kallman, K. D. (1962): Population genetics of the gynogenetic teleost, Mollinnesia formosa (Girard). *Evolution* 16 (4) : 497-504.
- Karaman, S. (1948): Prilog herpetologiji sjeverne Srbije. *Prirodoslovna Istraživanja* 24 : 51-74.
- Kawamura, T. and M. Nishioka (1979): Isolating mechanisms among the water frog species distributed in the Palearctic region. *Mitteilungen aus dem zoologischen Museum in Berlin* 55 (1) : 171-185 + Pl. VII-XII.
- Knudsen, K. and J. J. Scheel (1975). Contribution to the systematics of European green frogs. *Bulletin de la Société zoologique de France* 100 (4) : 677-679.
- Kyriakopoulou-Sklavounou, P. (1980): Plasma albumin patterns of the species Rana ridibunda in Greece. *Experientia* 36 (10) : 1171-1172.
- Lác, J. (1959 a): Príspevok k systematike, rozšíreniu a k bionómii skokanov Rana ridibunda Pall. a Rana esculenta L. na Slovensku. I. *Biológia, Bratislava*, 14 (9) : 665-673.
- Lác, J. (1959 b): Príspevok k systematike, rozšíreniu a k bionómii skokanov Rana ridibunda Pall. a Rana esculenta L. na Slovensku. II. *Biológia, Bratislava*, 14 (12) : 896-912.
- Leslie, J. F. (1979): Genetic studies on sexually and clonally inherited genomes of Poeciliopsis (Pisces: Poeciliidae). Ph. D. Thesis, New Brunswick, New Jersey : XI + 141 pp.
- Leslie, J. F. and R. C. Vrijenhoek (1978): Genetic dissection of clonally inherited genomes of Poeciliopsis. I. Linkage analysis and preliminary assessment of deleterious gene loads. *Genetics* 90 : 801-811.
- Leslie, J. F. and R. C. Vrijenhoek (1980): Consideration of Muller's ratchet mechanism through studies of genetic linkage and genomic compatibilities in clonally reproducing Poeciliopsis. *Evolution* 34 (6) : 1105-1115.
- Lewontin, R. C. (1974): The genetic basis of evolutionary change. Columbia University Press, New York and London : 346 pp.
- Macgregor, H. C. and T. M. Uzzell (1964): Gynogenesis in salamanders related to Ambystoma jeffersonianum. *Science* 143 (3610) : 1043-1045.

- Markert, C. L. (1963): Lactate dehydrogenase isozymes: Dissociation and recombination of subunits. *Science* 140 (3573) : 1329-1330.
- Markert C. L. (Ed.) (1975): *Isozymes. IV. Genetics and evolution*. Academic Press, New York, San Francisco and London : XXII + 965 pp.
- Maslin, T. P. (1962): All-female species of the lizard genus Cnemidophorus, Teiidae. *Science* 135 (3499) : 212-213.
- Maslin, T. P. (1968): Taxonomic problems in parthenogenetic vertebrates. *Systematic Zoology* 17 : 219-231.
- Maslin, T. P. (1971): Parthenogenesis in reptiles. *American Zoologist* 11 : 361-380.
- Maynard Smith, J. (1978): *The evolution of sex*. Cambridge University Press, Cambridge : X + 222 pp.
- Mayr, E. (1963): *Animal species and evolution*. Belknap Press, Cambridge (Massachusetts) : XIV + 797 pp.
- McKay, F. E. (1971): Behavioral aspects of population dynamics in unisexual-bisexual Poeciliopsis (Pisces: Poeciliidae). *Ecology* 52 (5) : 778-790.
- McKinney, C. O., F. R. Kay and R. A. Anderson (1973): A new all-female species of the genus Cnemidophorus. *Herpetologica* 29 : 361-366.
- Miller, R. R. and R. J. Schultz (1959): All-female strains of the teleost fishes of the genus Poeciliopsis. *Science* 130 (3389) : 1656-1657.
- Moore, W. S. (1975): Stability of small unisexual-bisexual populations of Poeciliopsis (Pisces: Poeciliidae). *Ecology* 56 (4) : 791-808.
- Moore, W. S. (1976): Components of fitness in the unisexual fish Poeciliopsis monacha-occidentalis. *Evolution* 30 (3) : 564-578.
- Moore, W. S. (1977): A histocompatibility analysis of inheritance in the unisexual fish Poeciliopsis 2 monacha-lucida. *Copeia* 1977 : 213-223.
- Moore, W. S. and F. E. McKay (1971): Coexistence in unisexual-bisexual species complexes of Poeciliopsis (Pisces: Poeciliidae). *Ecology* 52 (5) : 791-799.
- Muldal, S. (1952): The chromosomes of the earthworms. I. The evolution of polyploidy. *Heredity* 6 (1) : 55-76.
- Muller, H. J. (1964): The relation of recombination to mutational advance. *Mutation Research* 1 : 2-9.
- Nei, M. (1970): Accumulation of nonfunctional genes in sheltered chromosomes. *American Naturalist* 104 : 211-222.
- Nei, M. (1972): Genetic distance between populations. *American Naturalist* 106 (949) : 283-292.
- Nei, M. (1973): The theory and estimation of genetic distance. In: Morton, N. E. (Ed.): *Genetic structure of populations*. University of Hawaii Press, Honolulu : 45-54.

- Nei, M. (1975): Molecular population genetics and evolution. *Frontiers of Biology* 40, North-Holland Publishing Company, Amsterdam and Oxford : XIII + 288 pp.
- Nevo, E. (1976): Adaptive strategies of genetic systems in constant and varying environments. In: Karlin, S. and E. Nevo (Ed.): *Population genetics and ecology*. Academic Press, New York, San Francisco and London : 141-158.
- Nevo, E. (1978): Genetic variation in natural populations: patterns and theory. *Theoretical Population Biology* 13 (1) : 121-177.
- Nevo, E. and S. Y. Yang (1979): Genetic structure and variation of marsh frog populations in Israel. *Israel Journal of Zoology* 28 : 55.
- Nishioka, M., H. Ohtani and M. Sumida (1980): Detection of chromosomes bearing the loci for seven kinds of proteins in Japanese pond frogs. *Scientific Report of the Laboratory for amphibian Biology Hiroshima University* 4 (2) : 127-184.
- Nishioka, M., H. Ueda and M. Sumida (1981 a): Enzymes of Rana nigromaculata, Rana brevipoda, reciprocal hybrids and auto- and allotriploids. *Scientific Report of the Laboratory for amphibian Biology Hiroshima University* 5 (4) : 89-105.
- Nishioka, M., H. Ueda and M. Sumida (1981 b): Genetic variation of five enzymes in Japanese pond frogs. *Scientific Report of the Laboratory for amphibian Biology Hiroshima University* 5 (5) : 107-153.
- Omodeo, P. (1952): Cariologia dei Lumbricidae. *Caryologia* 4 (2) : 173-275.
- Omodeo, P. (1955): Cariologia dei Lumbricidae. II contributo. *Caryologia* 8 (1) : 135-178.
- Peters, G. (1971): Die intragenerischen Gruppen und die Phylogenese der Schmetterlingsagamen (Agamidae: Leiolepis). *Zoologische Jahrbücher (Systematik)* 98 : 11-130.
- Parker, E. D. and R. K. Selander (1976): The organization of genetic diversity in the parthenogenetic lizard Cnemidophorus tesselatus. *Genetics* 84 : 791-805.
- Pfannenstiel, M. (1954): Die Schwankungen des Mittelmeerspiegels als Folge der Eiszeiten. *Freiburger Universität Reden (neue Folge)* 18 : 1-19.
- Powell, J. R. (1975): Protein variation in natural populations of animals. In: Dobzhansky, T., M. K. Hecht and W. C. Steere (Ed.): *Evolutionary Biology* 1 : 79-120. Plenum Press, New York.
- Rogers, J. S. (1972): Measures of genetic similarity and genetic distance. *Studies in Genetics VII*, University of Texas Publication 7213 : 145-153.

- Schultz, R. J. (1969): Hybridization, unisexuality, and polyploidy in the teleost Poeciliopsis (Poeciliidae) and other vertebrates. *American Naturalist* 103 (934) : 605-619.
- Schultz, R. J. (1971): Special adaptive problems associated with unisexual fishes. *American Zoologist* 11 : 351-360.
- Schultz, R. J. (1977): Evolution and ecology of unisexual fishes. In: Hecht, M. K., W. C. Steere and B. Wallace (Ed.): *Evolutionary Biology* 10 : 277-331. Plenum Press, New York and London.
- Selander, R. K. (1976): Genic variation in natural populations. In: Ayala, F. J. (Ed.): *Molecular evolution*. Sinauer Associates, Sunderland (Massachusetts) : 21-45.
- Shaklee, J. B. (1975): The role of subunit interactions in the genesis of non-binomial lactate dehydrogenase isozyme distributions. In: Markert, C. L. (Ed.): *Isozymes*. Vol. I. Molecular structure. Academic Press, London : 101-118.
- Shaw, C. R. and R. Prasad (1970): Starch gel electrophoresis of enzymes - a compilation of recipes. *Biochemical Genetics* 4 (2) : 297-320.
- Sneath, P. H. A. and R. R. Sokal (1973): Numerical taxonomy. The principles and practice of numerical classification. W. H. Freeman and Company, San Francisco : XV + 573 pp.
- Thibault, R. E. (1974): Genetics of cannibalism in viviparous fish and its relationship to population density. *Nature* 251 : 138-140.
- Tunner, H. G. (1970): Das Serumeiweissbild einheimischer Wasserfrösche und der Hybridcharakter von Rana esculenta. *Verhandlungsberichte der deutschen zoologischen Gesellschaft* 64 : 352-358.
- Tunner, H. G. (1972): Serologische und morphologische Untersuchungen zur Frage der Artabgrenzung bei Wasserfröschen aus der Umgebung von Mainz (Rhein-Main-Gebiet). *Zeitschrift für zoologische Systematik und Evolutionsforschung* 10 (2) : 127-132.
- Tunner, H. G. (1973 a): Demonstration of the hybrid origin of the common green frog Rana esculenta L. *Naturwissenschaften* 60 (10) : 481-482.
- Tunner, H. G. (1973 b): Das Albumin und andere Bluteiweiße bei Rana ridibunda Pallas, Rana lessonae Camerano, Rana esculenta Linné und deren Hybriden. *Zeitschrift für zoologische Systematik und Evolutionsforschung* 11 (3) : 219-233.
- Tunner, H. G. (1974): Die klonale Struktur einer Wasserfroschpopulation. *Zeitschrift für zoologische Systematik und Evolutionsforschung* 12 (4) : 309-314.

- Tunner, H. G. (1979): The inheritance of morphology and electrophoretic markers from homotypic crosses of the hybridogenetic Rana esculenta. Mitteilungen aus dem zoologischen Museum in Berlin 55 (1) : 89-109.
- Tunner, H. G. (1980): Kreuzungsexperimente mit Wasserfröschen aus österreichischen und polnischen Mischpopulationen (Rana lessonae + Rana esculenta). Eine Analyse biochemischer und morphologischer Merkmale. Zeitschrift für zoologische Systematik und Evolutionsforschung 18 (4) : 257-297.
- Tunner, H. G. and M.-Th. Dobrowsky (1976): Zur morphologischen, serologischen und enzymologischen Differenzierung von Rana lessonae und der hybridogenetischen Rana esculenta aus dem Seewinkel und dem Neusiedlersee (Österreich, Burgenland). Zoologischer Anzeiger 197 (1/2) : 6-22.
- Tunner, H. G. and S. Heppich (1981): Premiotic genome exclusion during oogenesis in the common edible frog, Rana esculenta. Naturwissenschaften 68 (4) : 207-208.
- Tunner, H. G. and S. Heppich (1982): A genetic analysis of water frogs from Greece: evidence for the existence of a cryptic species. Zeitschrift für zoologische Systematik und Evolutionslehre 20 (3) : 209-223.
- Tunner, H. G. and H. Nopp (1979): Heterosis in the common European waterfrog. Naturwissenschaften 66 : 268-269.
- Tunner, H. G. and T. Uzzell (1974): Das Serumalbumin bei Rana ridibunda perezi (Salientia, Ranidae). Salamandra 10 (3/4) : 137-139.
- Uzzell, T. (1963): Natural triploidy in salamanders related to Ambystoma jeffersonianum. Science 139 (3550) : 113-115.
- Uzzell, T. (1964): Relations of the diploid and triploid species of the Ambystoma jeffersonianum complex (Amphibia, Caudata). Copeia 1964 (2) : 257-300.
- Uzzell, T. (1970): Meiotic mechanisms of naturally occurring unisexual vertebrates. American Naturalist 104 (938) : 433-445.
- Uzzell, T. (1979 a): Immunological distances between the serum albumins of Rana ridibunda and Rana lessonae. Proceedings of the Academy of Natural Sciences of Philadelphia 130 [1978] : 1-10.
- Uzzell, T. (1979 b): Immunological variation in Rana lessonae (Amphibia, Salientia). Mitteilungen aus dem zoologischen Museum in Berlin 55 (1) : 29-33.
- Uzzell, T. (1982 a): Immunological relationship of western Palearctic water frogs (Salientia: Ranidae). Amphibia Reptilia 3 (2/3) : 135-143.
- Uzzell, T. (1982 b): Introgression and stabilization in western Palearctic species of water frogs. In: Mossakowski, D. and G. Roth (Ed.): Environ-

- mental adaptation and evolution. Gustav Fischer, Stuttgart and New York : 275-295.
- Uzzell, T. (in press): An immunological survey of Italian water frogs. *Herpetologica*.
- Uzzell, T. and L. Berger (1975): Electrophoretic phenotypes of Rana ridibunda, Rana lessonae, and their hybridogenetic associate, Rana esculenta. *Proceedings of the Academy of Natural Sciences of Philadelphia* 127 (2) : 13-24.
- Uzzell, T., L. Berger and R. Günther (1975): Diploid and triploid progeny from a diploid female of Rana esculenta (Amphibia Salientia). *Proceedings of the Academy of Natural Sciences of Philadelphia* 127 (11) : 81-91.
- Uzzell, T. and I. S. Darevsky (1975): Biochemical evidence for the hybrid origin of the parthenogenetic species of the Lacerta saxicola complex (Sauria: Lacertidae), with a discussion of some ecological and evolutionary implications. *Copeia* 1975 (2) : 204-222.
- Uzzell, T., R. Günther and L. Berger (1977): Rana ridibunda and Rana esculenta: a leaky hybridogenetic system (Amphibia Salientia). *Proceedings of the Academy of Natural Sciences of Philadelphia* 128 (9) : 147-171.
- Uzzell, T. and H. Hotz (1979): Electrophoretic and morphological evidence for two forms of green frogs (Rana esculenta complex) in peninsular Italy (Amphibia, Salientia). *Mitteilungen aus dem zoologischen Museum in Berlin* 55 (1) : 13-27.
- Uzzell, T., H. Hotz and L. Berger (1980): Genome exclusion in gametogenesis by an interspecific Rana hybrid: evidence from electrophoresis of individual oocytes. *Journal of experimental Zoology* 214 (3) : 251-259.
- Uzzell, T. and H. G. Tunner (in press): An immunological analysis of Spanish and French water frogs. *Journal of Herpetology*.
- Vanzolini, P. E. (1970): Unisexual Cnemidophorus lemniscatus in the Amazonas valley: a preliminary note (Sauria, Teiidae). *Papeis avulsos de Zoologia* 23 : 63-68.
- Vogel, P. (1977): Isozyme der Lactatdehydrogenase (LDH) im Rana esculenta-Komplex. Dissertation Universität Zürich : 95 pp.
- Vogel, P. and P. S. Chen (1976): Genetic control of LDH isozymes in the Rana esculenta complex. *Experientia* 32 (3) : 304-307.
- Vogel, P. and P. S. Chen (1977): A further study of LDH isozymes in the Rana esculenta complex. *Experientia* 33 (10) : 1285-1287.
- Vrijenhoek, R. C., R. A. Angus and R. J. Schultz (1977): Variation and heterozygosity in sexually vs. clonally reproducing populations of Poeciliopsis. *Evolution* 31 (4) : 767-781.

- Vrijenhoek, R. C., R. A. Angus and R. J. Schultz (1978): Variation and clonal structure in a unisexual fish. *American Naturalist* 112 (983) : 41-55.
- Vrijenhoek, R. C. and R. J. Schultz (1974): Evolution of a trihybrid unisexual fish (Poeciliopsis, Poeciliidae). *Evolution* 28 (2) : 306-319.
- White, M. J. D. (1970): Heterozygosity and genetic polymorphism in parthenogenetic animals. In: Hecht, M. K. and W. C. Steere (Ed.): *Essays in evolution and genetics in honor of Theodosius Dobzhansky*. Appleton-Century-Crofts, New York : 237-262.
- White, M. J. D. (1978): *Modes of speciation*. W. H. Freeman and Company, San Francisco : VIII + 455 pp.
- White, M. J. D. (1980): Meiotic mechanisms in a parthenogenetic grasshopper species and its hybrids with related bisexual species. *Genetica* 52/53 : 379-383.
- Wickbom, T. (1945): Cytological studies on Dipnoi, Urodela, Anura and Emys. *Hereditas* 31 : 241-346.
- Wijnands, H. E. J. (1977): Distribution and habitat of Rana esculenta complex in the Netherlands. *Netherlands Journal of Zoology* 27 (3) : 277-286.
- Wijnands, H. E. J. (1978): Plasma albumins and biometrical characteristics of different forms of Rana esculenta complex. *Zoologische Jahrbücher (Systematik)* 105 : 337-346.
- Wijnands, H. E. J. and J. J. van Gelder (1976): Biometrical and serological evidence for the occurrence of three phenotypes of green frogs (Rana esculenta complex) in the Netherlands. *Netherlands Journal of Zoology* 26 (3) : 414-424.
- Williams, G. C. (1975): *Sex and evolution*. Princeton University Press, Princeton (New Jersey) : X + 200 pp.
- Wright, D. A. and F. H. Moyer (1966): Parental influences on lactate dehydrogenase in the early development of hybrid frogs in the genus Rana. *Journal of experimental Zoology* 163 (2) : 215-229.
- Wright, J. W. and C. H. Lowe (1968): Weeds, polyploids, parthenogenesis, and the geographical and ecological distribution of all-female species of Cnemidophorus. *Copeia* 1968 : 128-138.
- Wright, S. (1978): *Evolution and the genetics of populations*. Volume 4. Variability within and among natural populations. University of Chicago Press, Chicago and London : X + 580 pp.
- Yang, S. Y. (1971): Laboratory techniques. In: Selander, R., M. H. Smith, S. Y. Yang, W. E. Johnson and J. B. Gentry: *Biochemical polymorphism*

and systematics in the genus Peromyscus. I. Variation in the old-field mouse (Peromyscus polionotus). Studies in Genetics VI, University of Texas Publications 7103 : 85-90.

CURRICULUM VITAE

Am 19. August 1948 wurde ich, Hansjürg Hotz, Bürger von Zürich, in Zürich geboren. Von 1955 bis 1961 besuchte ich die Primarschule in Zürich, von 1961 bis 1967 das Realgymnasium Zürichberg, das ich im Herbst 1967 mit der Matura Typ B abschloss.

Von 1967 bis 1974 studierte ich an der Philosophischen Fakultät II der Universität Zürich; 1974 erwarb ich das Diplom als Naturwissenschaftler mit dem Hauptfach Zoologie und den Nebenfächern Mathematik, Geographie, Paläontologie, Geologie und Allgemeine Botanik. Während meines Studiums besuchte ich Vorlesungen und Kurse bei den Dozenten E. Batschelet, J. Biegert, G. Bächli, O. Bär, H. Boesch, H. Bolli, E. Brock, H. Burla, P. S. Chen, H. Conradin, C. D. K. Cook, A. Crnković-Fiorelli, E. Dal Vesco, H.-R. Dürrenmatt, K. v. Fischer, A. Gansser, G. Gensler, E. Hadorn, P. Hagemann, E. Hauschteck-Jungen, H. Hediger, H. Heusser, K. Hsü, K. A. Hünemann, H. Jungen, K. H. Kaiser, E. Kubli, E. Kuhn-Schnyder, H. Kummer, E. Leuzinger, H. Müller, R. Nöthiger, D. Rast, H. Rieber, O. Rohweder, H. Rüst, M. Schüepp, B. Stüssi, K. Suter, P. Tardent, E. Thomas, H. Tobler, R. Trümpy, D. v. Uslar, B. L. van der Waerden, G. Wagner, M. Waldmeier, H. Wanner, R. Wehner, C. Weissmann, H. Wysling, P. Yodzis und V. Ziswiler.

Seit 1979 arbeitete ich am Zoologischen Museum der Universität Zürich an der vorliegenden Dissertation unter der Leitung von Prof. Dr. H. Burla und Prof. Dr. T. Uzzell (Philadelphia, USA). Seit 1979 bin ich als Lehrbeauftragter der Philosophischen Fakultät II an der Universität Zürich tätig.

